VOLUME LXX NUMBER 4

THE
BOTANICAL GAZETTE

OCTOBER 1920

PHYSIOLOGICAL ISOLATION BY LOW TEMPERATURE IN BRYOPHYLLUM[1]

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(WITH SIX FIGURES)

Introduction

It has long been known that certain relations of dominance or control and subordination exist between different parts of plants. Among these relations the most general is the dominance of the chief growing tip over other buds, branches, etc.; that is, over other growing tips. That these relations are not specific for particular parts of the plant is shown by the fact that the growth and development of a bud, for example, may be inhibited by the chief growing tip of the plant, by a branch, or by a leaf. Moreover, the fact has been established that the dominance of one part over another is associated in some way with the metabolic activity of the dominant part. When this is inhibited, for example, by inclosure of a growing tip in an atmosphere without oxygen, or in plaster, the effect on other buds is essentially the same as if the growing tip had been removed; that is, it is no longer dominant, but may regain its dominance when the inhibiting conditions are removed and it again becomes active.

In various publications (CHILD 1–6) it has been pointed out that this relation of dominance and subordination is not peculiar to plants, but that similar relations exist in animals. In these

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and other papers it has also been maintained on the basis of various lines of evidence that this relation originates in a difference in rate of fundamental metabolic reactions, such differences of course being associated with differences in protoplasmic condition. These differences appear in the form of gradations in physiological condition, which have been called metabolic or physiological gradients. Since a discussion of the nature and origin of these gradients and the evidence on which the conclusions are based has recently appeared (CHILD **6**), extended discussion of these matters is not necessary at this time. In such a physiological gradient the dominant region is primarily the region of highest metabolic rate, of greatest physiological activity. The evidence indicates further that the relation between dominant and subordinate parts is primarily transmissive, rather than transportative in character; that is, that the dominance of a particular part is primarily dependent upon dynamic changes transmitted from it to other parts, rather than upon the transportation from the one part to the other of substances in mass. Since this dynamic effect of a region of high metabolic rate upon other parts is a factor in determining the metabolic rate of the parts affected, and since in protoplasm without highly specialized conducting paths it decreases with increasing distance from the region of high rate, the result of the establishment of such a region of high rate, however brought about, is the development of a more or less definite physiological gradient. The physiological axis in its simplest terms is such a gradient, determined in relation to a region of high activity which is the dominant region of the axis.

It has been found, largely through the work of HYMAN and BELLAMY, which is not yet published, that gradients in electrical potential and electric currents resulting from them are characteristic features of these physiological gradients. Discussion of the significance of these bioelectric currents is impossible here, but many facts make it highly probable that they are the factors primarily concerned in transmission of excitation (LILLIE **8–13**), and that they play a fundamental part in determining and maintaining the physiological gradients which arise in relation to regions of high metabolic rate. If this is true, the dominance of

one part over another is probably primarily a matter of the differences in electric potential and resulting currents. In general, the "high end," the dominant region of a physiological gradient, is externally electro-negative to other levels of the gradient, and in this respect it is similar to a region of excitation, which is also externally electro-negative to less excited or unexcited regions. From this viewpoint the physiological gradient may be regarded, at least tentatively, as the physiological expression or effect of the potential gradient and the resulting currents which arise in relation to a region of high metabolic rate. In fact, the physiological gradient in its simple form shows all the characteristics of an excitation-transmission gradient in protoplasm. In all except the simplest animals a nervous system with definite morphological conducting paths develops as an expression and resultant of the physiological gradients, and after definite nervous relations are established between parts, the dominance of a particular region, for example, the head, is no longer necessarily dependent upon the persistence of the metabolic conditions which originally determine its dominance. While the rate of metabolism concerned in the initiation of a nervous impulse is undoubtedly high, its total amount may be exceedingly small, yet the impulse may determine an enormous amount of metabolism in the organ affected by it.

In plants, however, no nervous system develops, and the relations of dominance and subordination apparently depend throughout life upon essentially quantitative physiological differences of the same sort as those in which the relation originates. The nervous structure of higher animals is capable of conducting impulses for long, perhaps for indefinite distances; but in the less highly specialized protoplasms of the simpler animals and the plants the dynamic effects of excitation undergo a decrement with increasing distance from their point of origin. Such a decrement determines the existence of the physiological gradient, and it is evident that under such conditions physiological dominance of any part must be limited in range, and that therefore the possibility of what the senior author has called physiological isolation (**1–6**), that is, of escape or isolation from such dominance without physical separation of parts, exists. Theoretically physiological isolation

may occur in four ways: first, growth in size of the organism may bring some part of it beyond the range of the dominant region; second, since dominance is primarily dependent upon the metabolic activity of the dominant region, a decrease in this activity, however brought about, will decrease the range of dominance and may bring about physiological isolation in more distant parts without increase in size; third, if physiological dominance is dependent upon transmission of electric or other dynamic effects through protoplasm, physiological isolation must result from blocking the passage of such effects; fourth, the subordinate part may be directly excited by external factors to such a degree that the action of the dominant part upon it is no longer effective, for example, stated in electrical terms, it may itself give rise to electric currents in the opposite direction from those in the dominant region and compensating them.

The physiologically isolated part behaves essentially as it would if the dominant part had been removed, or it itself separated from the dominant region. If its growth and development have previously been inhibited, it begins to grow and develop. If it represented a differentiated part of the body, as in many animals, it reacts in the simpler forms by losing this differentiation and may give rise agamically to a new individual.

Physiological isolation and consequent development of new parts or individuals as a result of growth is a familiar phenomenon in both plants and animals. The experimental decrease in the activity of the growing tip by inclosing it in an atmosphere lacking oxygen, or in plaster, as well as many cases of the inhibiting action of external factors in nature on growing tips afford numerous examples among plants of the second kind of physiological isolation. The fourth type of isolation appears in cases in which a bud may be made to grow in spite of the inhibiting action of a growing tip or other part, by subjecting it to external conditions which increase its activity. Such isolation may be brought about in some plants, particularly in the buds farthest away from the dominant region. In *Bryophyllum*, for example, the buds in the notches of the lower leaves will often develop under favorable external conditions.

As regards the third type of physiological isolation, by the blocking of passage of the action or effect, whatever its nature, less is known. McCallum (**21**) obtained some results along this line in plants by means of local anaesthesia, and it is a familiar fact that the passage of the nerve impulse can be blocked by an anaesthetized or a cooled region, as well as by various other means. For a long time one of us has had in mind experimentation along this line with plants, and Loeb's (**14–20**) recent work on *Bryophyllum* constituted an additional stimulus. Finally, in 1918–1919, experimentation was begun in the attempt to determine whether physiological isolation could be brought about by a local reversible action on some part of the connecting path between dominant and subordinate parts. Since it seemed desirable to avoid the use of anaesthetics or other chemical agents which might enter the plant tissues and be transported in one direction or another, low temperature was used as the blocking factor.

Method and apparatus

The low temperature was obtained by a current of cold water flowing through a block tin pipe of $\frac{1}{8}$ or $\frac{3}{16}$ inch inside diameter, this pipe being bent into loops or coils of the proper diameter and length to surround the portion of the plant to be cooled. In this way various lengths of stem, from 1 cm. to a whole internode or more, could be cooled. In preparation of the plant, the region to be subjected to the low temperature was first wrapped in tinfoil, and the loop or coil of pipe, supported by clamps attached to ring stands, was fitted about it in such manner that it was not in direct contact with the plant at any point, the space between stem and pipe being usually 0.5–1 cm. This space was then packed lightly with moistened absorbent cotton to keep the temperature as constant as possible, and finally the whole coil was wrapped closely in non-absorbent cotton to protect it from the outside temperature. In many experiments a thermometer was also inserted in the coil, but it was found that so long as the temperature of the water passing through the coil remained constant there was practically no change inside. In a part of the experiments the temperature of the water used was maintained in a tank by

means of a refrigerating brine coil under thermostatic control. The freezing point of the water in the tank was slightly lowered by the addition of alcohol in order to avoid accumulation of ice about the brine coil. The circulation of water from and to the tank was maintained by an electrically driven pump. The flow from the tank was led to a horizontal feeder pipe 6 ft. above the experimental table, and in this feeder six outlets were tapped about 30 cm. apart. At the table level a similarly tapped collector pipe returned the water to the tank. The flow for each plant was led from the feeder by one of the six insulated outlets, through rubber tubing to the loop or coil about the plant, through the coil and back to a corresponding tap on the collector pipe. With this apparatus as many plants as there were pairs of outlets (supply and return) could be placed under experimental conditions at the same time. Each supply pipe was fitted with a valve, making it possible independently to regulate or stop entirely the flow of cold water through any of the six coils. By means of these valves temperatures differing by several degrees could be maintained in different lines without affecting appreciably the temperature of the general supply. All exposed metal piping was covered with non-conducting material. During the winter months, while the temperature of the city water was so low that it could be used directly, a second similar system was also arranged for use with the city water. Each system was supplied with six connecting lines, so that twelve experiments could be conducted at the same time. With a little care it was possible to control within about 1° C. the temperature to which the cooled region was subjected. This temperature apparatus was devised by the junior author, and for its maintenance in proper working order during the experiments he is largely responsible.

The temperatures found to be effective for the purpose ranged from 2.5 to 6° C., according to the species of plant used and the region subjected to cooling. In the bean seedling, in which regions of the main stem were cooled, the more basal levels of the stem required a lower temperature than more apical levels to bring about physiological isolation and outgrowth of buds below the cooled zone.

Thus far experiments have been made chiefly with three species of plants: *Bryophyllum calycinum*, in which physiological isolation and outgrowth of buds in the notches of the leaves was brought about by cooling a region of the petiole; seedlings of *Phaseolus multiflorus*, the scarlet runner bean, in which isolation and outgrowth of axillary buds were brought about by cooling a region of the main stem between the buds to be isolated and the chief tip of the plant; *Saxifraga sarmentosa*, in which the isolation and development of the runner tip into a new plant was brought about by cooling a zone of the runner below its tip. A brief report of the results of these experiments has already appeared (CHILD and BELLAMY 7). The present paper is devoted to the experiments on *Bryophyllum*.

Experiments

The individual plants used ranged from 0.6 to 1.3 m. in height. Leaves from the upper half of the plant were selected for experiment in nearly all cases, particularly in the larger, older plants, in which the lower leaves are often in poor condition, or, when the plants have been kept in moist air, show more or less outgrowth of the buds during the winter in the intact plants. Various preliminary experiments were performed in order to determine to what extent physiological isolation might be brought about by external conditions acting directly on the leaves while still attached to the intact plant. By arranging bowls or jars containing water on ring stands about the plant in such a way that particular leaves were more or less completely submerged, it was found that at least during the winter and early spring months some of the leaf buds would grow in some cases, but to a greater extent on leaves at lower than on those at higher levels of the plant.

Again, a sudden rise in temperature from 15 to 25° C. in saturated atmosphere would usually induce outgrowth of some buds on leaves of the middle and lower levels of the plant, but not on those near the main tip. Such outgrowth usually consisted only of roots, and these were often inhibited after a few days.

Direct injury to the petiole, for example, compression by a screw clamp or by a cut partially through the petiole, was usually

effective, if sufficient in degree, in inducing more or less outgrowth of leaf buds, provided the leaf concerned was in saturated air or water. Slight superficial injury, even though it extended completely around the petiole, had little or no effect. An injury to the petiole of one leaf sufficient to induce outgrowth of some of the buds of that leaf usually induced outgrowth of some buds on the opposite leaf, and sometimes also on leaves of the next node above or below, if these were in moist air or water. Such mechanical injury by partial section of the petiole or by compression, however, was less effective than low temperature, unless the injury was sufficient to interrupt physiological continuity to a very considerable degree. Cuts half way through the petiole, for example, brought about development of some buds on the leaf, but usually of only a few, and in the case of lateral cuts the buds growing were not necessarily on the same side as the cut. Mechanical compression of the petiole by a screw clamp gave similar results. Fig. 1 shows a case in which the petiole of the leaf *A* was compressed to half its thickness by the screw clamp. The leaf *A*, the opposite leaf *B*, and one leaf *C* of the next pair below were partially submerged. On leaf *A* five buds grew out of eighteen submerged; on leaf *B*, seven out of seventeen submerged, but they are less advanced than those of *A*. On leaf *C* there is slight growth of four out of seventeen buds submerged, but this growth consists merely of short roots and is not visible in the figure. In another similar experiment slightly less compression resulted in outgrowth of only three buds out of twelve submerged on the leaf with compressed petiole, and no growth in *B* and *C*. Comparison of these results with those obtained by low temperature described later

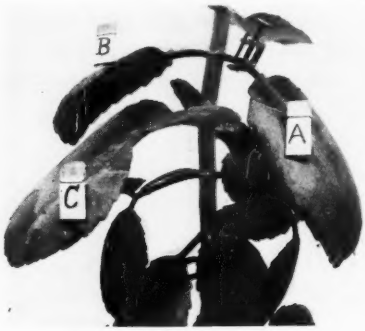

FIG. 1

shows that the low temperature is far more effective, even though no visible injury results, than mechanical compression.

These various experiments show very clearly that the dominance of the chief growing tip of *Bryophyllum* may be overcome to some extent at levels below the most apical five or six nodes without separating the leaf from the plant or inhibiting the chief tip, and in some cases by merely placing the leaf in water or in moist air with its petiole and attachment intact. Such isolation, however, usually results in development of only a part or a few, often of only one or two of the buds on a leaf. Whether physiological isolation of the leaf buds will occur as readily during the summer months has not yet been determined. The fact that injury to the petiole of one leaf may, if sufficient in degree, induce growth of buds in the opposite leaf and often in the leaves of adjoining nodes, shows further that the inhibition of growth of buds in any leaf is due, not merely to the chief tip of the plant, but to the opposite leaf and to some extent to other leaves also. This fact has also been shown by earlier work and more recently by LOEB's experiments.

The results of cooling a zone of the petiole, however, are much more striking. In these experiments a portion of the petiole 2–3 cm. in length is subjected to the low temperature, the rest of the petiole and leaf being exposed to room temperature, and the leaf blade more or less completely submerged in water, as indicated in fig. 2, or by placing an open bowl of water in such position that the leaf rests in the water. The succulent tissues of *Bryophyllum* are very susceptible to injury by continued pressure, and care must be taken that the low temperature coil does not touch the petiole, that the packing of the coil is not too tight, and that the petiole does not touch the edge of the jar or bowl in which the leaf is submerged. In some experiments, particularly the earlier, injury of the petiole resulted from one of these causes; but although the results of the experiments on mechanical injury indicate that the slight injuries thus produced had little or no effect on the leaf buds, only those experiments in which no visible mechanical injury of the petiole was found after removal of the low temperature zone are regarded as entirely satisfactory.

In room temperature of 20–25° C., growth of the leaf buds usually became visible three to four days after the low temperature coil was placed, the first indication being the outgrowth of one or

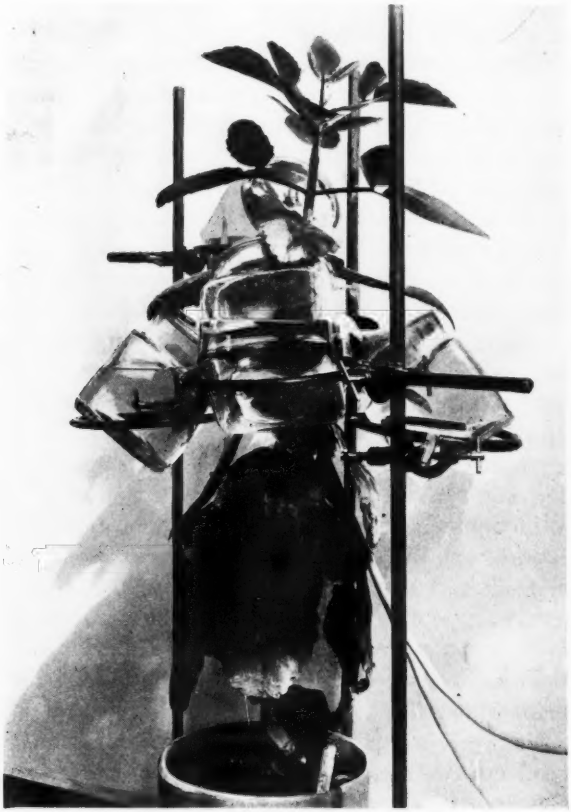

FIG. 2

more roots. If the low temperature coil was removed at this time, shoots usually did not appear, or appeared in only a few notches, the others being inhibited. If the low temperature coil remained in place six to eight days, shoots as well as roots were

usually clearly visible before its removal, and growth always continued afterward in at least a considerable number of notches. When the coil remained on the petiole for ten days or more, death of some of the epidermal cells usually occurred, although internally the petiole might be completely intact and to all appearances in good condition. Probably such superficial injuries were without effect, but cases in which they occurred were not regarded as conclusive.

In all, twenty-eight low temperature experiments were performed with *Bryophyllum* leaves attached to the plant. In fifteen of these there was some injury to the petiole, but in the other thirteen no injury was apparent. In all these experiments not only the experimental leaf, but the opposite leaf, and usually one or more leaves of nodes above and below the node of the experimental leaf, were more or less completely submerged, in order to determine to what extent these leaves were also affected. Usually all, or nearly all, buds on the submerged portion of the experimental leaf and in most cases those of the leaf opposite developed. On some leaves individual buds had been killed or injured by plant lice, from which the plant could not be kept entirely free. As far as possible, leaves were selected for experiment on which all the buds were apparently intact, but occasionally such buds failed to grow.

In order to determine whether cooling of a zone of the petiole stopped the flow of water to the leaf, experiments were performed in which the experimental leaf remained in air of medium humidity, instead of being submerged. Such leaves did not wilt, but remained fresh and in good condition, while leaves separated from the plant and exposed to the same atmosphere showed distinct wilting in the course of a few days. Evidently the cooled zone does not appreciably affect the flow of fluids to the leaf. The following descriptions and figures of typical experiments will serve to show the results attained.

Series 35, March 12, 1919.—Temperature of 2.5–3° C. was placed on petiole of one leaf of the eighth pair below the tip. The terminal leaflet of this leaf, of the opposite leaf, and one leaf of the ninth pair were submerged. Growth was visible in both leaves

of the eighth pair after three days, but none in the leaf of the ninth pair. After five days the cooled zone was gradually brought to room temperature and the coil removed. Fig. 3 shows the condition of the plant twelve days after the beginning of the experiment. The experimental leaf is the member on the left of the figure of the pair showing bud development. In this and the opposite leaf all buds which were submerged show vigorous outgrowth. In the leaf of the ninth pair ten buds out of twelve submerged developed, but only three of these produced distinct shoots, and all are much retarded as compared with those of the leaves above. Fig. 3 does not show the roots on this leaf, since they are on the under side.

FIG. 3

Series 44, March 30, 1919.—Temperature of 3–4° C. on petiole of one leaf (*A*) of seventh pair below tip. Opposite leaf (*B*) and both leaves (*C* and *D*) of sixth pair in water. The leaf *D* was in water eight days preceding the temperature experiment, but during that time showed no development of buds. After seven days the cooled zone was gradually brought to room temperature and the coil removed. The experimental leaf (*A*) showed growth in ten notches, *B* in two notches, *C* in none, and *D* in five notches. During the following week three days of high temperature in the greenhouse occurred, and this may have aided the development of some further buds. The condition of the four leaves eighteen days after the beginning of the experiment is indicated in fig. 4. In *A* every bud submerged has developed; in *B* nine buds out of fourteen submerged have developed, but only three of them have gone beyond the earliest stages; in *C* eight buds out of sixteen submerged show some development, but all except two have been inhibited in early stages; in *D* four buds out of fifteen submerged have developed

and show shoots as well as roots. Fig. 4 shows clearly the difference between *A* and the other leaves, although it does not show all the development in *B*, *C*, and *D*. The more advanced development of the four buds in *D* suggests that the eight days in water preceding the temperature experiment may have had some slight effect in the way of isolation, although it did not lead to visible development.

Series 45, April 4, 1919.—Temperature 2.5–3° C. on one leaf (*A*) of sixth pair below tip; *B*, opposite leaf, *C*, one leaf of seventh pair, *D*, one leaf of eighth pair, and *E*, one leaf of fifth pair also in water. After seven days the cooled zone was gradually brought to room temperature and the coil removed. Fig. 5 shows the plant seventeen days after beginning of the experiment. In *A* fourteen buds out of fifteen submerged developed and formed vigorous shoots; in *B* seven out of fifteen submerged developed to some extent, but produced only roots; in *C* eleven out of fifteen submerged showed some development, but only two produced shoots; *D* showed no growth; in *E* all buds submerged (thirteen) developed, but only six produced shoots. The growth on leaf *C* is not well shown in the figure. The slight development in the leaf *B* of this experiment is unusual. In most cases the mate of the experimental leaf shows almost or quite as much growth as the experimental leaf itself.

FIG. 4

Series 43, March 27, 1909.—Temperature of 2.5–3 C. on petiole of one leaf (*A*) of eighth pair; only the terminal leaflet in water; *B*, leaf opposite *A*, *C*, one leaf of eleventh pair, *D*, *E*, terminal leaflets of leaves of ninth pair, *F*, one leaf of seventh pair, *G*, one leaf of fifth pair, all in water. After six days the cooled zone was gradually brought to room temperature and the coil removed.

At this time *A* showed the greatest development, both as to stage and number of buds (eight) developing; *B* showed two buds developing; *C* and *D* none; *E* six; *F* one; and *G* none. Fig. 6 shows the plant fifteen days after beginning of the experiment. At this time in *A* seventeen buds out of nineteen had developed, and sixteen had produced shoots as well as roots; in *B* fourteen buds out of sixteen submerged had developed, thirteen with both shoots and roots; in *C* four buds out of nineteen submerged had developed, all with both shoots and roots; in *D* two buds out of ten

FIG. 5

FIG. 6

submerged had developed, one with shoot and roots; in *E* ten buds out of sixteen submerged had developed, all with shoots and roots; in *F* eight buds out of sixteen submerged had developed, three shoots and roots, five roots only; in *G* seven buds out of twelve submerged showed some slight development, but only two showed shoots, the others roots only.

In this and other experiments described, some of the isolations on leaves distant from the experimental leaf may not be the result of the cooling, but merely such as occur on most plants at this season, when leaves are submerged. In all the series, however, it is evident that the greatest degree of isolation, both as regards

number of buds developing and degree of development, has occurred in the experimental leaf and the leaf opposite.

In these experiments generally the buds which do not develop distinct shoots as well as roots within a week, either do not develop shoots at all, or give rise to shoots which grow very slowly and often soon cease to grow. Such buds are evidently inhibited to a greater or less degree. Apparently they represent somewhat less active or weaker buds, which react less rapidly than others to the isolating conditions, and so do not advance far enough before the return of the experimental leaf to normal conditions to maintain their growth afterward.

It appears true also that in general buds give rise only to roots when the degree of physiological isolation is relatively slight, while with more complete isolation shoots as well as roots appear. The length of the isolation period is probably also a factor, since the outgrowth of roots begins somewhat earlier than that of the shoots, or at least occurs more rapidly during the early stages. A brief period of isolation gives time for roots to appear, but a longer period is apparently necessary for the shoot to become well started.

Discussion

It is evident from these experiments that a mere cooling of a zone of the petiole of the *Bryophyllum* leaf without visible physical injury serves to block the inhibiting action of the chief growing tip and other parts upon the buds of that leaf, and also the inhibiting action of the leaf upon the buds of the opposite leaf and other leaves in the same region of the plant. That this cooling does not block the flow of fluids and substances in solution through the vascular bundles is indicated by the experimental fact noted that leaves show no wilting in an atmosphere in which leaves severed from the plant wilt. In the case of the bean seedling, to be discussed in a later paper, this is still more clearly evident, for there the zone of low temperature is placed about the main stem, and all substances passing from below to those parts of the plant above the cooled zone must of course pass through it. In such cases there is no wilting of the parts above the zone, and growth is either not at all inhibited, or, when the region cooled is young

and not fully developed, growth of the parts above may be retarded slightly for two or three days, but soon proceeds normally. These facts do not support the view which LOEB has advanced that the inhibiting action of the growing tip and of other parts on buds is due to the transportation of inhibiting substances through the regular channels of transportation in the plant. In these experiments such transportation is not appreciably or only very slightly affected, yet the zone of low temperature is much more effective as a means of physiological isolation than mechanical compression or partial section of the petiole, except when these involve the greater part of the petiole tissues. Unless we assume that the hypothetical inhibiting substance in some way is rendered inactive by the short cooled zone, we must conclude that the dominance of the growing tip and of other regions over a particular leaf is not dependent upon the flow of substances through the vascular bundles to the leaf, but rather upon some sort of action which is dependent upon the physiological activity of the cells. When this activity is inhibited by the low temperature, the action is blocked, unless and until some degree of acclimation of the cooled zone occurs. Such acclimation occurs very readily in the bean seedling, and in many cases a temperature which at first serves as a block becomes ineffective after a few days. In short, the experiments indicate that the physiological dominance of one region over another in these plants is dependent on some sort of effect transmitted physiologically through the living active protoplasm, rather than upon substances transported by the flow of fluids.

LOEB appears not to distinguish clearly two different aspects of the relations of parts: the one which is concerned with the conditions that prevent or permit the initiation of development and growth in a subordinate part; the other which is concerned with the amount of growth or development of the part which may occur after its initiation. Nutritive factors may play a large part in determining the amount of growth of buds, but there are no reasons for and many against maintaining, as LOEB did in earlier papers, that they initiate it. Again, the mass of shoots and roots developing from an isolated *Bryophyllum* leaf may show a certain proportion to the size of the leaf, since the amount of certain

nutritive substances available must depend upon the size of the leaf, but such relation tells us nothing concerning the factors which initiate the development.

On the other hand, the assumption of the transportation of inhibiting substances, made in LOEB's later papers, also involves certain difficulties. In the first place, each part which produces such substance or substances must be immune to the action of the substance which it produces, since it is not inhibited by it, yet in the case of growing tips the substance produced by one growing tip inhibits other tips. This presupposes a remarkable specificity of action on the one hand and absence of specificity on the other, and it is difficult to conceive how the hypothetical substance could possess the properties required. Certain assumptions concerning the direction of flow of the inhibiting substances also have no basis in fact and do not agree well with the facts at hand. Certain other objections to the assumption of inhibiting substances scarcely require discussion in view of the work of various botanists and the experiments just described.

In an early paper LOEB (**14**, pp. 251–253) endeavored to show that isolation is not the initiating factor in the outgrowth of buds on the leaf of *Bryophyllum*, and described three experiments to prove his point (see his *figs. 1, 2, 3*). In the first a leaf partially submerged is completely separated from the stem; in the second it remains attached to a piece of stem cut off above and below the node and the opposite leaf is removed, but its axillary bud remains; and in the third the opposite leaf also remains. In the first experiment and in the third the submerged buds develop, in the second they do not, but the axillary bud of the opposite side develops in the absence of its leaf. LOEB maintains that the leaf in the second experiment is more isolated than in the third, but its buds do not grow out, therefore isolation cannot be the factor determining the development of the buds. This conclusion is incorrect and based upon a misconception of isolation. Actually the leaf of the second experiment is less isolated than in the first and third, because in this experiment the axillary bud of the opposite side develops and inhibits the leaf buds. If this growing tip is removed, the buds of the leaf will develop. In the third experiment the

axillary bud is inhibited by its own leaf and there are no active growing tips to inhibit the direct action of the water in inducing bud development in the experimental leaf. Isolation from an active growing tip is the chief factor in the development of the leaf buds, and such isolation exists to a greater degree in LOEB's first and third experiments than in the second, in which the axillary bud of the opposite side starts before the leaf buds of the experimental side. These three experiments, therefore, instead of disproving, as LOEB asserts, that isolation is the factor initiating development of the leaf buds, constitute evidence in support of the conclusion that it is such a factor. Moreover, according to LOEB's later assumption of inhibiting substances, it would seem that isolation must be the initiating factor.

The chief results of the paper are summarized as follows. The cooling of a zone of the petiole of the *Bryophyllum* leaf to a temperature of 2.5 to 4° C. for a few days is a very effective means of inducing the outgrowth of the leaf buds. Usually the opposite leaf and often leaves of adjoining nodes also show more or less development. The passage of fluids to the leaf is not appreciably interfered with by the cooled zone; therefore it seems improbable that physiological isolation of the leaf can be due to the blocking of passage of inhibiting substances transported in these fluids.

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SWELLING OF AGAR IN SOLUTIONS OF AMINO ACIDS AND RELATED COMPOUNDS

D. T. MacDougal and H. A. Spoehr

(with six figures)

A study of the behavior of a large variety of substances in aqueous solution on the hydration of agar has shown that there are very few such solutions in which agar swells to a greater degree than it does in distilled water. There are some substances, however, which increase the hydration of agar above that attained in pure water. These are the amino acids. The amino compounds are of such immediate biological importance that a discussion of their action deserves special consideration, and may aid in explaining the scattered results obtained by various workers in which increased total growth and apparently catalytically accelerated actions have been obtained by the addition of certain amino acids to culture solutions.

The purification of the agar, as well as the preparation of the agar plates and the instruments and methods for measuring the swelling, have already been described in detail by MacDougal.[1] The results here discussed were obtained by the application of these methods. The amount of swelling in water is taken as the standard. Thus the percentage of swelling of the dried plates in water is expressed as 100, and that in the various solutions is calculated on this basis.

Swelling of agar in solutions of amino acids

The dried agar plate, prepared from the specially purified agar and used in this experiment, showed a total swelling in distilled water of 2000 per cent. The amino acids, glycocoll, alanin, and phenylalanin were used in 0.01 normal concentration. The solutions in which the agar plates were allowed to swell were renewed every 24 hours; the swellings were complete after about 6 days. The results are given in table I.

[1] MacDougal, D. T., Auxographic measurement of swelling of biocolloids and of plants. Bot. Gaz. **70**: 126–136. 1920.

In table II are given the results of some earlier experiments made with plates prepared from the "bacto-agar" of the Digestive Ferments Company. The swellings in the solutions of the corresponding organic acids are also given for comparison. Thus it can be seen that in acetic acid agar exhibits a much lower swelling than in the a-amino compound, glycocoll. The same relation is maintained with the other acids.

TABLE I

SWELLING OF AGAR PLATES 0.11 MM. IN THICKNESS AT 15°C. IN 0.01 NORMAL SOLUTIONS OF AMINO ACIDS, CALCULATED ON BASIS OF SWELLING IN WATER TAKEN AS 100; SOLUTIONS RENEWED EVERY 24 HOURS; SWELLING OF AGAR PLATES IN WATER 2000 PER CENT

Water	Glycocoll	Alanin	Phenylalanin
100.....	165	151	161

TABLE II

SWELLING OF AGAR PLATES 0.10–0.23 MM. IN THICKNESS AT 16–17°C. IN SOLUTIONS OF AMINO ACIDS AND CORRESPONDING ORGANIC ACIDS, CALCULATED ON BASIS OF SWELLING IN WATER TAKEN AS 100; DRIED PLATES SWELLED 2570 PER CENT IN WATER; DURATION OF SWELLING 20–60 HOURS, DURING WHICH TIME SOLUTIONS WERE NOT RENEWED

Normal concentration	Water	Glycocoll	Acetic acid	Alanin	Propionic acid	Asparagine	Aspartic acid	Succinic acid
0.01.....	100	115	61	94	51	91	49	49
0.002.....	100	123	59	108	63	94	58	62
0.0004.....	100	103	76	.....	70	106	69	68
0.00008.....	100	.....	102	.....	.....	126	81	98

It is worthy of note that agar behaves quite differently from gelatine in relation to acids and bases, and that this also applies to the amino acids, as shown in table III. Agar is a carbohydrate, and as such exhibits some of the properties of an exceedingly weak acid. DR. MCGEE of this laboratory determined by means of the indicator method[2] that in 0.75 per cent solution the purified agar shows a hydrogen ion concentration expressed by $P_H = 6.5$.

[2] DUGGAR, B. M., The use of the colorimeter in the indicator method of H ion determination with biological fluids. Ann. Mo. Bot. Gard. **6**:61–70. 1918.

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It has long been known that carbohydrates form salts with metals, and that they react with the chlorides, sulphates, and other salts of the heavy metals, such as copper, lead, mercury, gold, iron, silver, etc., to form the corresponding carbohydrate salt with

TABLE III

SWELLING OF DRIED GELATINE PLATES 0.5 MM. IN THICKNESS AT 16–17°C.; SWELLING IN WATER 600 PER CENT

Normal concentration	Water	Propionic acid	Alanin
0.1	100	.....	91
0.05	100	256	83
0.01	100	185	88
0.002	100	130	86
0.004	100	83	75

the liberation of hydrochloric, sulphuric, or other acids. McGEE has determined the hydrogen ion concentration of a number of heavy metal salts in 2 per cent aqueous solution, and also in mixtures of 2 per cent of the salts plus 2 per cent d-glucose. A 2 per cent solution of d-glucose in water showed $P_H = 6.6$.

TABLE IV

HYDROGEN ION CONCENTRATION EXPRESSED AS P_H OF 2 PER CENT SOLUTIONS OF SOME HEAVY METAL SALTS AND OF SAME MIXED WITH 2 PER CENT D-GLUCOSE

$ZnCl_2$	6.3	$AgNO_3$	5.5	$HgCl_2$	3.9	$CuSO_4$	4.6
$ZnCl_2$ + glucose ...	5.4	$AgNO_3$ + glucose...	5.3	$HgCl_2$ + glucose...	3.8	$CuSO_4$ + glucose...	4.4

It is apparent that in the addition of the glucose to the heavy metal salts the acidity of the mixture is appreciably raised.

The amino acids being amphoteric electrolytes, it is to be expected that they would behave like acids toward bases, and like bases toward acids. Furthermore, there are a number of reactions of which the amino acids are capable which may be of importance in interpreting their behavior toward agar. Thus the simplest amino acid, glycocoll (NH_2CH_2COOH), can apparently give rise to an internal salt, NH_3CH_2COO. Glycocoll in solution would then exist in equilibrium as the un-ionized

NH_2CH_2COOH together with its ions and with NH_3CH_2COO, as well as a hydrated form $OHNH_3CH_2COOH$. The existence of this latter compound has been used to explain why acids such as glycocoll do not follow the simple Ostwald dilution law. The recent observations of BIRCKNER[3] on the interaction of ethyl alcohol and certain amino acids may be interpreted in favor of the theory of salt formation of the alcohol with the amino group. Further insight as to whether the increased swelling of agar in amino acids is due to simple salt formation or to the formation of a compound related to the form $OHNH_3CH_2COOH$ was sought in a study of the behavior of agar toward ammonium hydroxide and some related substances.

Swelling of agar in alkaline hydroxides and in ammonium salts

A study of the behavior of agar in solutions of various alkaline hydroxides revealed a number of facts worthy of notice. The first experiments were conducted in the usual manner with 25 cc. of the hydroxide solutions in the dishes containing the pieces of agar. An examination of the auxograph record of the swelling thus obtained showed a remarkable similarity, in that after about 24 hours there was a marked acceleration in the rate of swelling, and the final results were very similar in all cases. In figs. 1 and 2 the curves of swelling are reproduced for potassium hydroxide and ammonium hydroxide. The total swellings thus obtained in the various solutions are given in table V.

TABLE V

SWELLING OF DRIED PLATES OF AGAR IN 25 CC. OF ALKALINE HYDROXIDE SOLUTIONS AT 15°C.; TOTAL SWELLING OF AGAR PLATES IN WATER 3950 PER CENT

Normal concentrations	Water	NH_4OH	LiOH	NaOH	KOH
0.01	100	63	60	55	49
0.001	100	77	77	75	81

Titrations after 24 hours of the solutions in which the agar had been swelling showed that the solutions had decreased

[3] BIRCKNER, V., Jour. Biochem. **38**:245–254. 1919.

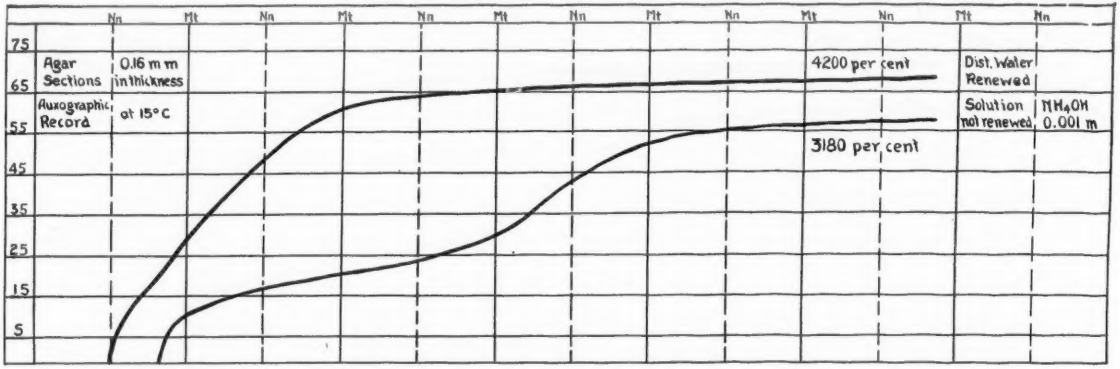

FIG. 1.—Auxographic record of swelling of agar plates in 0.001N solution of NH_4OH not renewed, and in water renewed every 24 hours

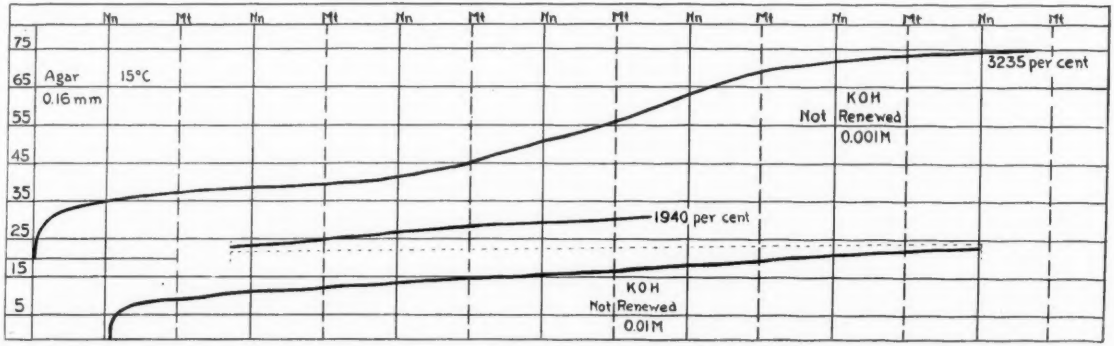

FIG. 2.—Auxographic records of swelling of agar plates in 0.01 and 0.001N solutions of KOH not renewed during course of experiment.

considerably in strength in this time, probably due to absorption of CO_2 from the air, and, in the case of the ammonium hydroxide, to volatilization. Experiments were then made in which the solutions were removed from the agar and replaced by fresh solutions every 12 or 24 hours. The results thus obtained differ radically from the previous ones, as to form of the record made by the swelling agar, as well as to the total amount of swelling attained in each case. Figs. 3 and 4 give the auxograph record of the swelling agar in solutions renewed every 12 hours. The relatively sudden increase in rate of swelling after 24 hours, which was so striking in the experiments in which the solutions were not renewed, is entirely absent. This accelerated swelling undoubtedly represents the rate of swelling in a solution which is but slightly alkaline and approaches that obtained in water. Furthermore, the total swelling of agar in KOH, NaOH and LiOH is decidedly lower in the solutions which had been renewed. Especially noteworthy, however, is the fact that ammonium hydroxide in 0.001 normal concentration produces a swelling considerably in excess of water when the solution is renewed. This observation has been verified repeatedly.

The differences in the swelling of agar in NH_4OH and $C_2H_5NH_2$ on the one hand, and in LiOH and KOH on the other hand, are considerable, particularly in the more dilute solutions.

TABLE VI

SWELLING OF DRIED AGAR PLATES AT 15°C. IN ALKALINE HYDROXIDE SOLUTIONS, RENEWED EVERY 12 HOURS; TOTAL SWELLING OF DRIED AGAR PLATE IN WATER 3950 PER CENT

Normal concentration	Water	NH_4OH	Ethyl amine	LiOH	NaOH	KOH
0.01	100	25	31	24	21	21
0.001	100	115	88	40	35	29

Owing to the fact that in solutions of ammonium hydroxide and ethyl amine there exist equilibria respectively between dissolved NH_3 and the hydroxide, and between dissolved $C_2H_5NH_2$ and its hydroxide, the condition in solutions of these substances, particularly in the more concentrated solutions, offers a rather complicated situation not dissimilar from that obtaining in solutions

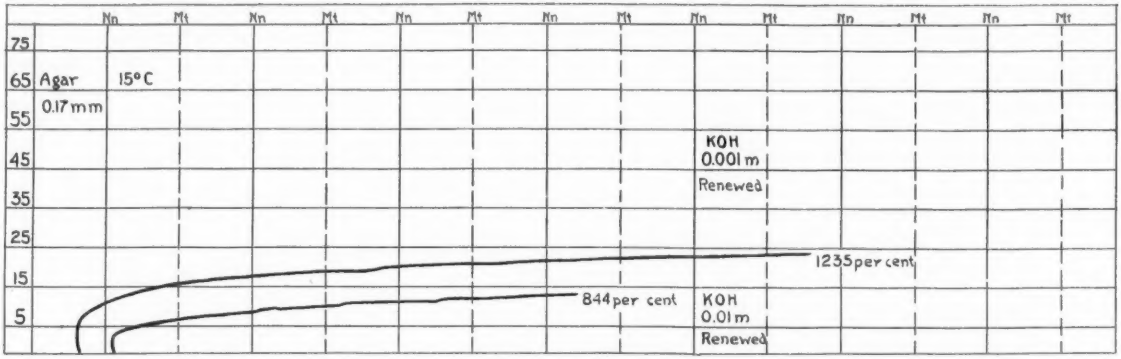

FIG. 3.—Auxographic records of swelling of agar plates in 0.01 and 0.001N solutions of KOH renewed every 12 hours

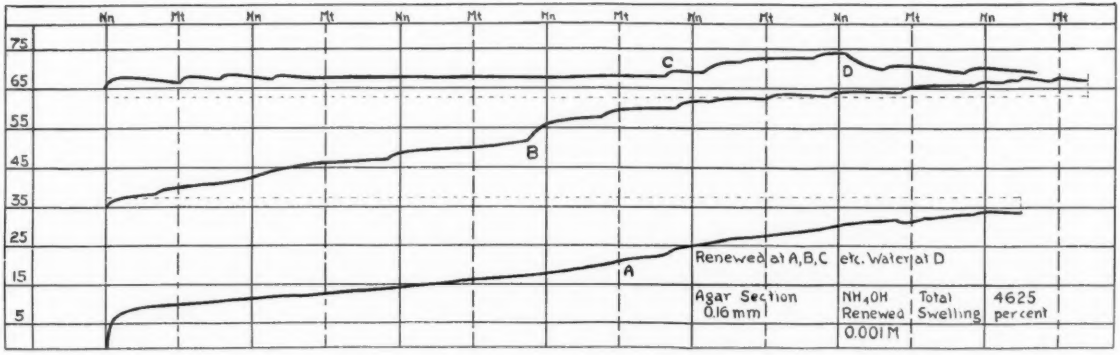

FIG. 4.—Auxographic record of swelling of agar plates in 0.001N solution of NH_4OH renewed frequently and replaced by water at *D*

of the amino acids. From these experiments it would appear that the stronger the base, as indicated by its position in the electromotive series, the less is the effect on the swelling of agar. Thus we have in effect K < Na < Li, and, in the weaker concentrations, ethyl amine, which is a stronger base than ammonium hydroxide, falling below this in hydration capacity. Interesting is the case of aniline, a very weak base. Separate experiments with this substance were carried out, in which agar showed the following swelling: in 0.01N solution, 110; in 0.001N solution, 100; in water, 100.

Further investigation of the effect of a possible agar-salt formation on the swelling was undertaken by a study of the behavior of dried agar plate which had been prepared from agar previously treated with KOH and NH_4OH. Portions of the purified agar were allowed to remain in such a quantity of 0.01 normal KOH and NH_4OH solutions as to make up a 2.5 per cent agar solution. After about 14 hours these solutions with the agar were heated, and the agar plates poured and then dried in the usual manner. The "kaliated" and "ammonated" plates thus prepared were then allowed to swell in solutions of KOH, NH_4OH, and distilled water. During the first 24 hours the water in which these plates had swelled became distinctly alkaline. Thereafter the water was renewed every 48 hours and showed but slight alkaline reaction. Thus the hydrogen ion concentration of the water in which the plates had been swelled, as determined by the indicator method, gave the values shown in table VII.

TABLE VII

Time	"Kaliated" plate P_H	"Ammonated" plate P_H
August 13	10.0	10.0
August 15	7.4	7.2
August 17	7.7	7.4

The figures given for August 15 and 17 probably indicate the values representing the products of hydrolysis of the "kaliated" and "ammonated" plates. The total swellings of these plates are given in table VIII.

From the results thus far obtained it would appear that the swelling of agar in water is surpassed only by that taking place

FIG. 4.—Auxographic record of swelling of agar plates in 0.001N solution of NH_4OH renewed frequently and replaced by water at *D*

under conditions favorable to the formation of a combination of agar with ammonia or its closely related compounds. The exact nature of these agar combinations and the manner in which water acts upon them have not as yet been determined with any degree

TABLE VIII

SWELLING OF DRIED "KALIATED" AND "AMMONATED" AGAR PLATES AT 15°C.; ACTUAL SWELLING IN WATER OF "KALIATED" PLATE 3450 PER CENT; "AMMONATED" PLATE 2450 PER CENT

Plate	Water	0.01 N KOH	0.001 N KOH	0.01 N NH_4OH	0.001 N NH_4OH
"Kaliated"	100	57	83	.....	.....
"Ammonated"	100	.....	.....	107	122

of satisfaction. Ammonium salts, such as ammonium chloride and ammonium acetate, in the concentrations thus far tried, have shown no augmenting effect on the swelling. Ammonium acetate, although neutral in water solution, is hydrolyzed to a considerable extent with the formation of the ions of ammonium and acetic acid. The effect of these salts, in which the nitrogen can be considered as fully satisfied, on the swelling of agar shows a typical neutral salt effect.

TABLE IX

SWELLING OF DRIED PLATES OF AGAR AT 15°C. IN SOLUTIONS OF AMMONIUM CHLORIDE AND AMMONIUM ACETATE; TOTAL SWELLING OF AGAR PLATES IN WATER 1700 PER CENT

Normal concentration	Water	NH_4Cl	$NH_4CO_2CH_3$
0.01	100	58	58
0.001	100	94	100

An examination of the records produced by swelling agar in which the solutions are regularly renewed indicates clearly that the addition of a fresh solution of ammonium hydroxide affects distinctly the subsequent swelling of the already partially hydrated agar (figs. 3, 4). Thus, for example, sections of agar had swelled to values in water = 100; in 0.01N NH_4OH = 25. After the sections had attained their full swelling of 25 in 0.01N NH_4OH, the hydroxide solution was replaced by water. The agar swelled further to a value equal to 73. Thereupon other sections of dried

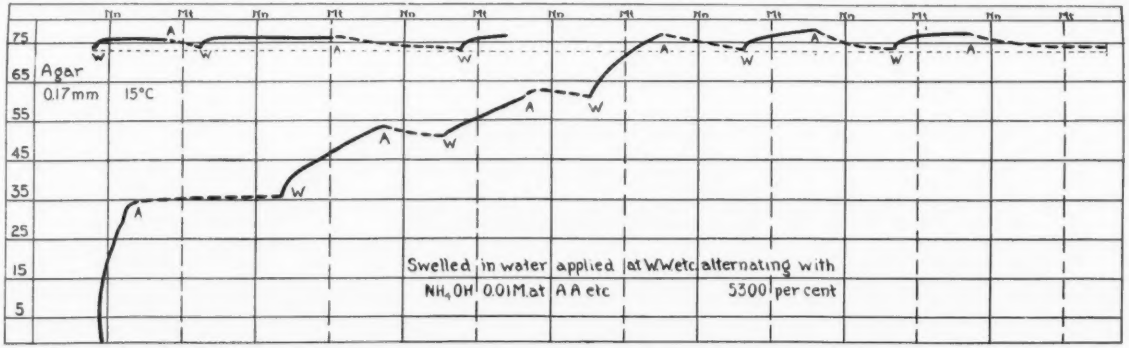

FIG. 5.—Auxographic record of swelling of agar plates in 0.01N solution of NH_4OH alternated every 24 hours with water; broken line indicating swelling in NH_4OH.

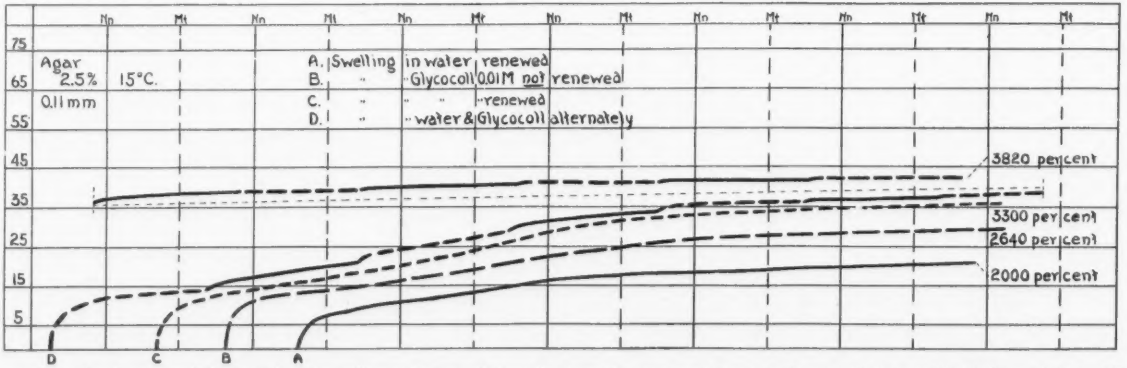

FIG. 6.—Auxographic records of swelling of agar plates: *A*, in water renewed every 12 hours; *B*, in 0.01N solution of glycocoll not renewed; *C*, in 0.01N solution of glycocoll renewed every 24 hours; *D*, water and 0.01N solution of glycocoll alternating; broken line indicating swelling in glycocoll.

agar plate were allowed to swell by alternating every 12 hours water and ammonium hydroxide in 0.01N solution. The curve for this swelling is reproduced in fig. 5. At first both the NH_4OH and the water cause swelling. As the hydration continues, however, the swelling in NH_4OH becomes slight, and finally there is an actual shrinkage in the hydroxide with, moreover, a subsequent swelling again in the water. The total swelling thus attained greatly exceeds that in water or ammonium hydroxide alone. The total swelling of the dried agar plates treated thus alternately with water and ammonium hydroxide was 5300 per cent, or on the basis of the swelling in water equal to 100, the swelling in alternating NH_4OH and H_2O equaled 134. Similar experiments were carried out in which glycocoll was alternated with water. Thus with 18 changes in 15 days agar swelled 191 as against 100 in water. In a solution of glycocoll which was not changed during the entire swelling the agar showed a swelling value of 132. The curves thus formed by the swelling of agar in glycocoll are shown in fig. 6.

No adequate explanation has been found for the augmenting effect on the swelling of agar produced by ammonia and the amino acids and here described. From a consideration of the relative effects of various hydroxides it would appear that something analogous to salt formation and subsequent hydrolysis of this compound may be involved.[4] Ammonium, as a weak base united with agar, an exceedingly weak acid, would form a salt which would very easily be hydrolyzed. Under conditions in which this hydrolysis is suppressed by the presence of a common ion (NH_4), the excessive swelling does not take place. Thus when dried agar plates are allowed to swell by alternating solutions of 0.001N, NH_4OH, and NH_4Cl the total swelling does not exceed that attained in water, but actually falls somewhat below that value.

The possible biological significance of these changes in volume, which are also exhibited by agar-protein mixtures with respect to growth and metabolism, seems to be so great as to warrant, not only this brief description, but also the formation of plans for the extension of the experiments.

DESERT LABORATORY
TUCSON, ARIZ.

[4] BRACEWELL, R. S., Jour. Amer. Chem. Soc. 41:1511–1515. 1919.

EARLY STEM ANATOMY OF TODEA BARBARA

JAMES E. CRIBBS

(WITH PLATES XXIII–XXVI)

The work of JEFFREY (6) and FAULL (4) on the Osmundaceae, in which they interpreted the stele as containing a medulla of cortical origin, has stimulated an investigation into the condition of the stele as found in the ancestry of the line, and has brought forth a great deal of criticism.

The present investigation of the early sporophyte stages of *Todea barbara* was undertaken to ascertain whether in the early periods of organization and development there might be some phases which would be significant as to the ancestral stelar condition. Spores were sown, and from the resultant gametophytes large numbers of sporophytes were secured and examined in all stages of development up to the time of departure of the fourteenth leaf trace. Considerable variation appears in the development of the young sporophyte and in many of its anatomical features. This is especially true of the young plant before it has established its independence of the gametophyte. The first root, for instance, sometimes elongates first, breaking through the old venter wall, but more commonly the leaf takes this initial step. There is irregularity in the phyllotaxy of the first leaves, in the appearance and attachment of the first roots, in the appearance of sclerenchyma, and in the time of medullation.

The earliest protoxylem to appear has been observed to specialize opposite the foot attachment, where it continues downward in one of the two protoxylem points of the root, and upward into the stem, where it is associated with the elements which are diverted into the first trace. Part of the protoxylem elements of the second group in the root turn out and enter the foot region, while the others are oblique and are continuous into the basal portion of the stem, or may terminate at the junction of the foot and stem,

forming triaxial tracheae which are in contact at the three points with the tissues of the three plant regions.

The young stem directly above the foot attachment contains xylem elements which are relatively short and grouped in a typical protostelic manner. They are surrounded by a single layer of nucleated parenchyma cells, external to which a few sieve tubes are disposed in a much broken circle. These are succeeded externally by a pericycle layer one cell in width, and which, like the phloem, is more or less discontinuous. Frequently an embayment occurs in the xylem at the edge of the foot attachment with the stele (fig. 6). This is occupied by parenchyma which is usually continuous with that of the root which lies between the protoxylem points, and extends up into the stem, where it usually becomes more shallow, and is exserted before the departure of the first leaf or soon thereafter. This indention, when present, occurs at right angles to the plane of the foot attachment and never in the position of a gap (fig. 32).

The first leaf trace is detached from the stele about 70–150 μ above the foot, and is preceded by the appearance of 1–3 parenchyma cells in the xylem, which assume an eccentric position beneath the place of leaf exit. Quite commonly these first cells are in contact laterally with the sheath parenchyma; but whether in contact below or not, they become confluent with the sheath parenchyma at the time of exit of the first trace, when the xylem of the stele once more forms a solid group. It is quite evident, therefore, that this first xylem parenchyma is merely an accompanying feature of the departure of a simple trace from the protostele. Very similar to the preceding is an unusual behavior observed when an embayment of parenchyma at the foot attachment became decurrent in the xylem of the root. This parenchyma cell could not be shown to be in contact with that surrounding the xylem, but apparently was lost in the midst of the metaxylem elements of the root, just as the decurrent parenchyma associated with the departure of the early traces in the stem may end blindly in the tracheae a short distance below.

DEBARY (3) considered the stele of the Osmundaceae as a sympodium of leaf traces, and, in reference to the young stem,

states: "The first bundle, which usually ends blind in the foot of the embryo, curves after a very short course through the stem into the first leaf; from the point of curvature the development of a bundle, which runs out into the second leaf, begins. In the case of the subsequent leaves the same conditions prevail." DEBARY'S interpretation of the stelar structures in this group has been supplanted by the theory of the stele as a unit in stem development (VAN TIEGHEM **12**), which was strongly supported by GWYNNE-VAUGHAN'S (**5**) work on *Primula*, and subsequently by others, and which has at present gained most general credence. The conditions found in the young stem of *Todea barbara* give evidence in support of the later theory; for, as is readily observed, the trachea group above the foot is composed of 15–25 elements, while the number entering the first leaf is commonly but four, and was never observed to exceed six (fig. 5).

The stem axis bends at each node in the young sporophyte, so that the traces depart from the outer angles, a behavior undoubtedly referable to the manner of origin of the leaves, and is caused by pressure of segments cut off from the leaf apical cells and stem apical cells following the isolation of the former in a small meristem which has not yet become closely invested with leaf bases (figs. 35, 37). In the early organization of the stem a single apical cell appears, which is usually of the three-sided pyramidal type. Sometimes it is truncated at the base, and in most instances was found to be broader near the middle than at the top (fig. 36). Frequently, however, the apical cell is four-sided in transverse section (figs. 39, 41), and, like that of the root, cuts off segments more or less irregularly, often giving the appearance of a cluster of initials (figs. 38, 46). Only one instance of initials not certainly referable to a single cell was found (fig. 40). This instance occurred after the organization of the medulla, and appears to be a true case of more than one apical cell; but the earliest meristems were always referable to a single initial. At the level of departure of the first trace there is still a paucity of sieve cells (fig. 5); the pericycle is likewise incompletely developed, and, in fact, the feeble development of all the extra-xylar elements is quite noticeable in contrast with their relative prominence at higher levels in the

stem. The trachea elements of the early stele are strikingly uniform in caliber, pointed at the ends, relatively short, and display no certain delimitation of protoxylem and metaxylem.

Above the point of separation of the first and subsequent leaf traces a groove commonly occurs in the xylem axis directly opposite the departing strand (figs. 7, 9, 11, 15). This is continuous upward a variable distance, where it gradually becomes more shallow and disappears. Not infrequently, however, after the first one or two traces have departed, this groove may detach or be connected internally with a single parenchyma cell which passes up in the center of the trachea group, where it occasionally ends blindly, but more commonly becomes continuous with the sheath parenchyma once more through the embayment of decurrent parenchyma associated with the departure of the succeeding trace. Frequently such central parenchyma cells are in continuity externally through lateral embayments at the edge of root attachments, similar to that which occasionally accompanies the attachment of the foot. A leaf trace sometimes departs from the stele, which includes parenchyma decurrent from the trace above, without being accompanied by a break in the xylem cylinder.

In fig. 8 two included parenchyma cells appear, which are in contact with the sheath above (fig. 9). The embayment occurring opposite the departure of the second trace in this instance does not close up, but becomes continuous with that which isolates the third trace (figs. 10, 11). Above the third trace the protostele becomes divided in the plane of the exit of the last trace (fig. 12), but, contrary to expectation, neither segment is exserted as a trace, but the succeeding trace is detached from the segment to the right at a higher level (fig. 13). The two remaining strands fuse almost immediately, and subsequently an indention occurs opposite the last trace (figs. 14, 15). This is continuous with a group of two parenchyma cells which becomes centrally located (fig. 16), and is in contact with the sheath parenchyma again at the departure of the fifth trace (fig. 17). Reference to this series shows that five leaves take their departure from the stem before the organization of the second root, which appears directly beneath and is associated with the sixth leaf (fig. 18). The earliest phyllotaxy

in this instance is expressed by the fraction $\frac{2}{5}$. It later changes to a $\frac{3}{8}$ arrangement, which appeared most frequently in the early stem.

A solid protostele occurs a short distance above the fifth trace, and is illustrative of a constant recurrence of a perfectly solid stele associated with the departure of either the fifth or sixth foliar strand (fig. 18). The appearance of the protostele at the detachment of the fifth trace is shown in fig. 28, which is typical of this level of the stem in all of the young plants studied. It will be noted that the xylem is composed of tracheae alone, although internal parenchyma was present both above and below the three preceding nodes, and is constantly present above the level of the sixth trace. Although the occurrence of internodal parenchyma is common in the young stele, the number of elements is usually very limited. Occasionally, however, the internodal pocket becomes quite extensive (fig. 1), when the central cylinder assumes the appearance of a true siphonostele with centrally placed thin-walled elements. Yet despite the siphonostelic aspect, the stem becomes distinctly protostelic at the node above, and also at the level of exit of the fifth and sixth traces. The parenchyma pocket shown in fig. 16 is quite the normal condition, and is seen to be a basipetal extension from the cleavage of the stele at the node above.

It has already been pointed out that the departure of a trace from the protostele may so influence the xylem that it becomes entirely divided into two segments. A recurrence of this is shown in fig. 19, in which instance, however, the indentions of both the fifth and sixth traces are concerned, affecting the stele from opposite sides. The two residual strands coalesce immediately above the node as in the former instance cited (fig. 12). The eighth trace takes its departure from the stele in a similar manner to the fifth, leaving a solitary intruded parenchyma cell which is soon replaced above by tracheae, leaving a solid protostele (fig. 20). Even at nodal regions the stele was always observed to contain thin-walled elements above the level of the eighth leaf, while more commonly the fifth or sixth node marks the upper limit of a stele entirely free from these elements.

There is a gradual increase in the number of xylem elements accompanying the enlargement of the stem up to the time of

medullation (figs. 22, 23). In the change from the protostele to a siphonostele there is a rapid increase in the diameter of the stem, and an appearance of parenchyma cells with included resinous storage similar to those of the pericycle. These increase rapidly in number with the appearance of a permanent medulla, but so far as observed no real sclerenchyma of the type found in the adult stele occurred in young stems up to the time of the separation of the fourteenth leaf trace (fig. 24). This early organization of the medulla takes place without the appearance of internal phloem, nor could sieve cells be demonstrated at any level in the central parenchymatous elements of the young stele. It was further observed that there was no endodermal invagination accompanying medullation, or the departure of traces from the young stele, up to that time. True leaf gaps occur in the stele after the appearance of the medulla, and the traces take their departure in a manner already described by SINNOT (11).

The storage parenchyma cells already mentioned as being very prominent in the early medulla have been observed to occur as early as the time of separation of the third trace, but more commonly about the time the sixth trace leaves the central cylinder. The first elements of this character are usually located opposite the place of exit of a trace and appear free in the sheath parenchyma (fig. 2), or occur at the edge of a root attachment. In a few instances they have proved to be continuous with like elements of the pericycle layer, the storage contents of which cells they resemble. The most pronounced intrusion of such cells observed in any of the young stems is shown in fig. 44, where they occur opposite a trace and at the edge of a root attachment. In no instance, however, has it been possible to demonstrate any thickenings on the walls of these elements such as occur on the endodermis, and it seems that their relation to the pericycle is not at all constant.

The endodermis is organized early in the development of the sporophyte, and is continuous over the early stem, the primary root, and the tissues which elongate and diverge into the foot. At a comparatively early stage it closes over the conductive elements in the foot, and thus entirely incloses the stele from the root apices to the meristematic region of the stem apex. A transverse section

of the foot in a three-leaved sporophyte is shown in fig. 26, and it will be noticed that it almost caps the foot tissues at this early stage, only two cells remaining free. Throughout all the stages, so far as studied in the young stem, there was no indication whatever of the endodermis dipping into the stele; but in complete continuity it passes over the gaps in the xylem caused by the departing leaf traces, without the slightest tendency to invaginate. A commonly recurring feature of the endodermis from about the level of the sixth leaf trace was the absence of storage materials opposite leaf gaps. Frequently there was likewise an absence of storage in the pericycle at the same point. Across this gap in the storage material, however, the endodermis was always found to be continuous.

The origin of the pericycle from the stem apex is difficult to determine, and it could not be definitely referred to initials which would point to a common origin with the endodermis. For the most part the pericycle cells contain an abundance of finely granular resinous material. As has already been pointed out, the pericycle does not form a complete circle in the earliest stages of the stele, but is interrupted at many points. At the level of the sixth or eighth trace it is rarely more than one cell thick, and in the young sporophyte it seldom exceeds two cells in thickness except at the edge of root attachments and in the adaxial angle of the leaf traces, where it frequently becomes very prominent (figs. 3, 44), and, like the endodermis, is quite regularly filled with finely granular material or with larger granules similar to those of the storage cells in the sheath parenchyma and medulla. The pericycle is increased in thickness by periclinal divisions (fig. 1), but the so-called "quergestrickten Zellen" of the older stem which have their origin in this way do not appear prominently in the earliest stages, but appear much more abundantly after the development of a central medulla.

There is a paucity of sieve cells in the lower levels of the stele, where they are most prominently and regularly developed on the outer edge of the xylem elements which are about to turn out from the cylinder as traces. The sieve cells are elongated elements terminated by oblique walls. The radial, terminal, and likewise the

tangential walls when in contact with similar cells are perforated with openings which vary from simple pits to relatively large sieve plates with numerous small apertures. The transversely elongated elements derived from the pericycle by tangential divisions are, as pointed out by SEWARD and FORD (10), sieve elements which are of later origin than the sieve tubes of the protophloem. The sieve plates of these elements, as asserted by FAULL (4), show callus plugs, and all the essential features of true sieve cells are present. There was no evidence of the appearance of phloem in the internal parenchyma in any of the material investigated, nor were the sieve cells ever observed to appear in the parenchyma tissue of the foliar gaps.

As already stated, there is a single row of nucleated parenchyma cells which surround the xylem in the first stages of the stele. At higher levels these elements increase in amount by tangential divisions. In longitudinal view they are observed to be narrow, elongated, with acutely oblique terminal walls. These resemble very closely the earliest xylem parenchyma. Are these elements to be considered as of cortical origin, as asserted by JEFFREY (7) and FAULL (4), or are they to be considered as distinctly stelar and representing undeveloped potential xylem elements differentiated by the meristematic stem apex? In the young stele these cells do not resemble the cortical tissues either in topography or cytology. The central ones at first have distinctly acute terminal walls, and in general topography are altogether like the tracheae, except, of course, for the secondary thickenings of their walls. At higher levels these internal unthickened elements frequently have transverse terminal walls or are but slightly obliqued from the horizontal. Further evidence for the stelar origin of these elements is found in the occurrence of tracheids in the same linear series with parenchyma cells above and below (fig. 29). It was further noticed in such instances that the tracheids were terminated by walls which were transverse or almost so, instead of having the strongly oblique prosenchymatous type so characteristic of the normal tracheae of the xylem axis. Cells of this type were found on the inner edge of the xylem in contact with the parenchyma, and in a few instances on the outer border where

they were in contact with the sheath parenchyma. A first supposition that these were protoxylem elements was readily disproved; for the cells are continuous up the stem axis, and in no instance turned out into the diverging xylem group of the leaf traces, as do the protoxylem cells. Their squared terminal walls are likewise not characteristic of protoxylem as found in the stele and traces. They always were observed to include some cytoplasm and most frequently a degenerate nucleus. A transverse view of these cells is shown in fig. 27, where it will be noted that they have a thickening equal to that of the normal tracheae, and in conjunction with the true xylem elements do not develop the lacunae between cell walls except occasionally, when it is barely distinguishable. They also lack the angle thickenings so characteristic of the Osmundaceae. In view of their belated appearance, their topographical and cytological features, they are considered as abnormal tracheae, arrested in development, and are considered as evidence indicative of stelar origin of the medulla by reduction. SEWARD and FORD (**10**) referred to similar elements found in *T. superba* and *T. hymenophylloides*, and KIDSTON and GWYNNE-VAUGHAN (**8**) interpreted them as probably being vestigial. That these are the last remnants in our living species of the peculiar central xylem now known to have been present in *Kalesskya* (**8**) and *Thamnopteris* (**9**), extinct protostelic members of this family belonging to the Upper Permian, seems entirely probable, and is the most logical interpretation.

The primary root of the sporophyte, as well as the roots which appear successively with the development of leaves in the early plants, are characteristically diarch (occasionally triarch), with radially arranged phloem. A narrow zone of parenchyma separates the xylem and phloem in a manner similar to that in the young stem stele (fig. 43). Following the development of the first root, two or more leaves regularly occur before the appearance of the second. This in most instances is associated with the third or fourth leaf, but in a few cases it has been observed to appear considerably later. In the series represented by figs. 6–24 it will be noted that the second root is associated with the sixth leaf. Roots, following their delayed appearance, frequently develop in an aberrant manner, forming often without any particular

relation to departing traces. Leaves frequently develop without corresponding roots. This early vacillating condition is replaced by regularity after the establishment of a central parenchyma, and a single root takes its departure from the stele just below the attachment of the leaf trace. Subsequently there is a second period of irregularity when either one or two roots are detached from the stele with each leaf.

In his studies of the apical regions of *Todea barbara*, BOWER (1) states: "The roots take their origin from a single cell of the endodermis which is situated opposite a xylem strand." In all cases observed in the young stem the root apical cell originated in the pericycle (figs. 45, 48). The initial cell enlarges, and, following a few radial divisions, a single apical cell develops which is quite variable in shape, but very early organizes into a three-sided pyramidal cell (fig. 47). BOWER (1) further states that of a number of root apices examined in *Todea barbara* "not one showed a clearly marked single apical cell. Some, however, showed somewhat irregular arrangements, and in some it appeared uncertain whether the meristem be referable to three or four initial cells. In the majority of the roots observed it is clearly referable to four initial cells, separated from each other by the four principal walls." In examining the root meristems in the young plants of *Todea barbara*, 28 single apical initials were recorded in as many different plants, and not a single instance of four initials was observed. There were a few cases which showed some variation from the pyramidal three-sided cell, with the divisions occurring unevenly. The segments from the apical cell are usually very large and do not divide immediately, resulting in a meristematic group resembling in longitudinal view a cluster of three or four initials. These, however, have always been referable in the root to a single initial which was prevailingly of the triangular-pyramidal variety. Thus the coaxial type described by BOWER (1) was not found in any of the root meristems of the early sporophytes.

The outer cortical cells of the primary root are usually the first to develop the sclerenchyma thickenings which later become so conspicuous a feature of the stem cortex. From the primary root they are extended to the adjoining cells of the stem in which

they persist throughout the axis. Frequently, however, the lower portion of the stem cortex is free from sclerenchyma, in which instance it is found to appear in the stem at the place of attachment of the second root, from the base of which it extends about the stele. When it first appears in this manner, the petioles of the leaves below the point of introduction most generally lack the characteristic cortical wall thickening of the cells surrounding the central bundle. After its first appearance in the stem it is sparingly developed or absent from the base of the next one or two petioles, although quite abundant in the stem axis and the leaves at higher levels, which would indicate that the rachis has been the last region of the plant to develop the sclerenchyma. An endophytic fungus was found to occur frequently in the cortical tissues of the root, external to the endodermis and internal to the sclerenchymatous cells of the peripheral region. It was found to gain entrance by way of root hairs, and also by dissolving its way through the epidermal cell wall at the edge of the root cap (fig. 42).

The first leaf originates at a point about 0.1 mm. above the foot attachment. The trace is isolated by an embayment, and carries off from the main axis about four cells (varies from three to six), which usually form a narrow band (fig. 5) which, once in the petiole, usually becomes endarch in its arrangement (fig. 33). Subsequent traces depart in a similar manner, carrying out an increasingly greater number of xylem elements until the appearance of a definite medulla tissue. The first petioles are practically wingless, but with an increase in leaf size and number they overlap and become conspicuously winged. The metaxylem elements meanwhile become more numerous, spreading out so as to form a semicircle with the opening toward the stele (fig. 34). The poorly defined phloem of the first trace becomes more definite and abundant in the second and third, where it may form a complete circle about the xylem.

In the petioles of the lower leaves the xylem sometimes assumes a distinctly mesarch arrangement, but is not completely invested by phloem (figs. 4, 25). The occurrence of primitive structures in the basal petioles has long been recognized, and the appearance of mesarch strands here is further evidence for the origin of our

present Osmundaceae from a protostelic group. It becomes particularly significant when we observe that *Kalesskya* and *Thamnopteris* were characterized by traces which departed in a protostelic manner and were strongly mesarch until they had entered for some distance into the petioles, where the xylem opened out and presented an appearance like that in the rachis of *Osmunda* and *Todea*. As already stated, these early representatives of the Osmundaceae were present in the Upper Permian and much preceded the Lower Cretaceous siphonostelic species *Osmundites skidegatensis* to which JEFFREY (7) referred for the most primitive type of osmundaceous stele available. The recurrence of mesarch strands, abnormal central tracheids, and a typical protostele in the young stem of *Todea* are interpreted as indicative of the descent of our present Osmundaceae from a protostelic ancestry.

Summary

1. The young sporophyte of *Todea barbara* is protostelic.

2. Preceding the departure of the early traces one or more parenchyma cells appear in the xylem group, or occupy a groove which is in contact with the sheath parenchyma. These, at the time of isolation of the trace from the central cylinder, become confluent with the xylem sheath, leaving the stele solid, or grooved opposite the strand. This depression is continuous up the stem, where it either becomes more shallow and is lost, or detaches one or more parenchyma elements which become centrally placed in the xylem group where they end blindly, or are in continuity with parenchyma decurrent from the succeeding trace.

3. The stem meristematic tissue is derived from a single apical cell of the triangular-pyramidal type. It, like the root initial, shows variation in the order of segmentation. Only one instance not certainly referable to a single initial was found.

4. Roots originate from the pericycle in the young sporophyte and very early develop a single apical cell which is broadly triangular and pyramidal, characterized by variation in shape and order of segmentation. Segments are large and frequently give the appearance of two or more apical initials. All root meristems examined, however, were referable to a single initial.

5. The cortical sclerenchyma is apparently of root origin, and is extended to the stem cortex from the primary root or from those which develop later in association with leaves.

6. An endophytic fungus is frequently found in the root cortex. It was observed to gain entrance through root hairs and through newly formed epidermis at the edge of the root cap.

7. A phyllotaxy represented by the fraction $\frac{3}{8}$ is most frequently found in the young plant. A $\frac{2}{5}$ arrangement was recorded in addition to a few cases of irregularity.

8. Leaf traces usually are endarch while in the stem cortex, and the protoxylem elements, which at first form a narrow band, spread out on the adaxial embayment which occurs in the metaxylem higher up in the petiole of the leaves.

9. Typical mesarch bundles have been observed in the petioles of early leaves. These are interpreted as being indicative of the ancestral condition, and in fact present the type of arrangement found by KIDSTON and GWYNNE-VAUGHAN to be characteristic of *Kalesskya* and *Thamnopteris*, Upper Permian representatives of this family.

10. The transversely elongated phloem elements are derived from periclinal divisions of the pericycle, and, as recorded by SEWARD and FORD, are sieve tubes which, in the organization of the stele, appear considerably later than the protophloem.

11. No instance of phloem within the medulla or central parenchyma was found at any level in the young stele.

12. The endodermis is continuous over the primary root, foot, and early stem. At no place in the young stem does it turn in through the gaps or embayments in the xylem.

13. Internal endodermis could not be demonstrated at any stage in the young stele.

14. The earliest parenchyma to appear within the xylem is composed of elements with pointed ends, whose caliber and length are very similar to the xylem elements, and conspicuously unlike the cortical cells.

15. No sclerenchyma was observed in the medulla up to the time of departure of the fourteenth leaf trace, although parenchyma cells with included resinous substances were quite abundant.

16. After the first organization of a definite medulla, short, thickened xylem cells have been observed to be present on the inner edge adjoining the pith. These elements, although thickened, were poorly lignified, contained some protoplasm and a degenerate nucleus, had transverse or slightly obliqued terminal walls, were usually in axial continuity above or below with parenchyma elements, and when in contact with adjoining tracheae did not develop the intercellular lacunae so characteristic of the protoxylem and metaxylem of this family. These elements are considered as belated xylem of vestigial character, and, because of their position and relation to the internal parenchyma, are evidence of medullary origin by stelar reduction. Cells of this type were not found in either the petiole or root.

17. The appearance of mesarch traces in basal leaves, a protostele in the early stem, the similarity in topography of early xylem parenchyma to xylem, the appearance on the inner border of the xylem of peculiar short tracheids with transverse terminal walls, the entire absence of internal phloem, the absence of internal endodermis, and the complete unity of the endodermis (which shows no indication of invagination) are all features in the ontogeny of *Todea barbara* which are indicative of a protostelic ancestry. These features assume a special significance when we can correlate them with similar structures in primitive forms, and their recurrence in *Kalesskya* and *Thamnopteris* further validates the theory of the protostelic origin of our living Osmundaceae.

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EXPLANATION OF PLATES XXIII–XXVI

PLATE XXIII

FIG. 1.—Transverse section of stele between third and fourth traces, showing extensive parenchyma pocket and periclinal divisions of pericycle; ×300.

FIG. 2.—Stele with tenth trace and seventh root; storage parenchyma in xylem sheath opposite trace, and three xylem parenchyma cells; ×88.

FIG. 3.—Transverse section of stele and seventh root; absence of storage in endodermis and pericycle opposite trace; gaps of sixth and seventh leaves; extensive pericycle development opposite sixth trace; ×138.

FIG. 4.—Mesarch grouping of xylem elements in petiole of third leaf, $\frac{1}{3}$ mm. above separation from stele; ×118.

FIG. 5.—Transverse section of stele and first trace, showing early phloem cells and paucity of extra-xylar elements in stelar cylinder; ×58.

PLATE XXIV

FIGS. 6–24 inclusive, ×40 diameter.

FIG. 6.—Foot attachment and lateral embayment of parenchyma.

FIG. 7.—First trace and grooving of xylem opposite.

FIG. 8.—Xylem with two parenchyma elements included.

FIG. 9.—Separation of second trace and grooving of xylem.

FIGS. 10, 11.—Separation of third trace from stele.

FIG. 12.—Cleavage of protostele into two strands.

FIG. 13.—Fourth trace being isolated from strand to right.

FIGS. 14–16.—Embayment and inclosure of parenchyma elements preliminary to detachment of fifth trace.

FIG. 17.—Connection of parenchyma of preceding figure with that of xylem sheath at time of separation of fifth trace.

FIG. 18.—Solid protostele above fifth trace; first root detached immediately below this level at lower left.

FIG. 19.—Second cleavage of protostele, with seventh trace to right.

FIG. 20.—Solid protostele with detached tracheae, decurrent from root which leaves stele immediately above.

FIG. 21.—Enlarged xylem group just before medullation.

FIG. 22.—Early medullation and inclusion of first storage parenchyma cells; eleventh trace.

FIG. 23.—Twelfth trace and eighth root.

FIG. 24.—Increase in storage parenchyma cells and medulla; ninth root.

PLATE XXV

FIG. 25.—Mesarch bundle in petiole of first leaf; ×625.

FIG. 26.—Transverse section of foot, showing closing of endodermis in three-leaved stage; ×88.

FIG. 27.—Transverse section of abnormal tracheids; ×625.

FIG. 28.—Stele at time of departure of fifth trace, showing solid xylem group and prominent sheath parenchyma; ×300.

FIG. 29.—Longitudinal section of tracheid in axial continuity with xylem parenchyma cells; ×300.

FIGS. 30, 31.—Sieve tubes with oblique terminal walls; ×300.

FIG. 32.—Stele immediately above foot; lateral embayment to right of foot attachment and indention of stele to isolate first trace at left; ×300.

FIG. 33.—Normal endarch bundle of first trace; ×88.

FIG. 34.—Fifth leaf strand 2.25 mm. above origin; phloem encircles xylem which shows adaxial embayment; ×300.

PLATE XXVI

FIG. 35.—Stem apical cell with leaf initial isolated at left; ×300.

FIG. 36.—Longitudinal view of stem apical cell; ×575.

FIG. 37.—Isolation of leaf initial from stem apical cell; ×575.

FIGS. 38, 39.—Irregularity in segmentation of stem apical cell; ×575.

FIG. 40.—Abnormal meristem with apparently four initials; ×300.

FIG. 41.—Transverse view of normal stem apical cell; ×129.

FIG. 42.—Cortical cell of root with endophytic fungus; ×625.

FIG. 43.—Transverse section of normal root, showing diarch character; ×88.

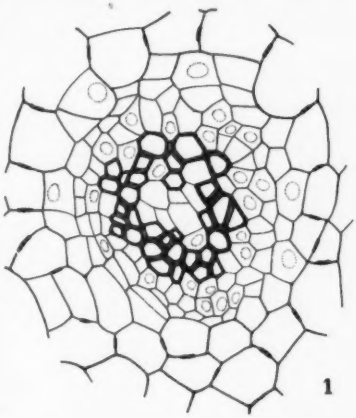
FIG. 44.—Transverse section of stem, showing at left a pericyclic intrusion and strong development at edge of root attachment; ×88.

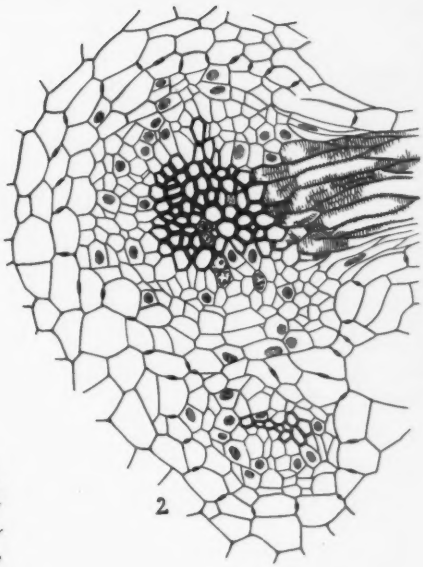
FIG. 45.—Longitudinal section of root apical initial in pericycle; ×300.

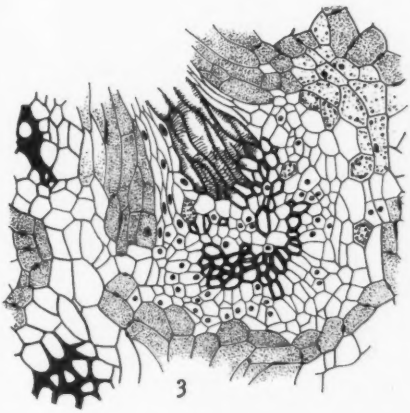
FIG. 46.—Irregular segmentation of root apical cell; ×575.

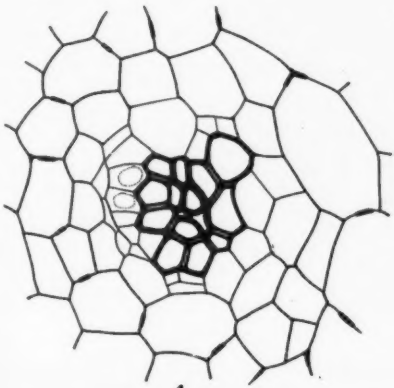
FIG. 47.—Transverse section of triangular-pyramidal root apical cell; ×300.

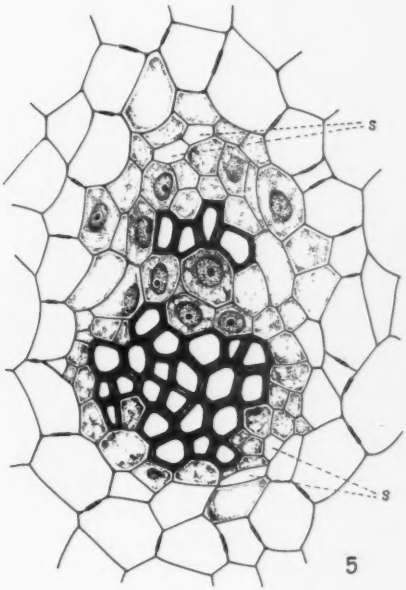
FIG. 48.—Tangential section of stele, showing organization of root apex in pericycle; ×300.











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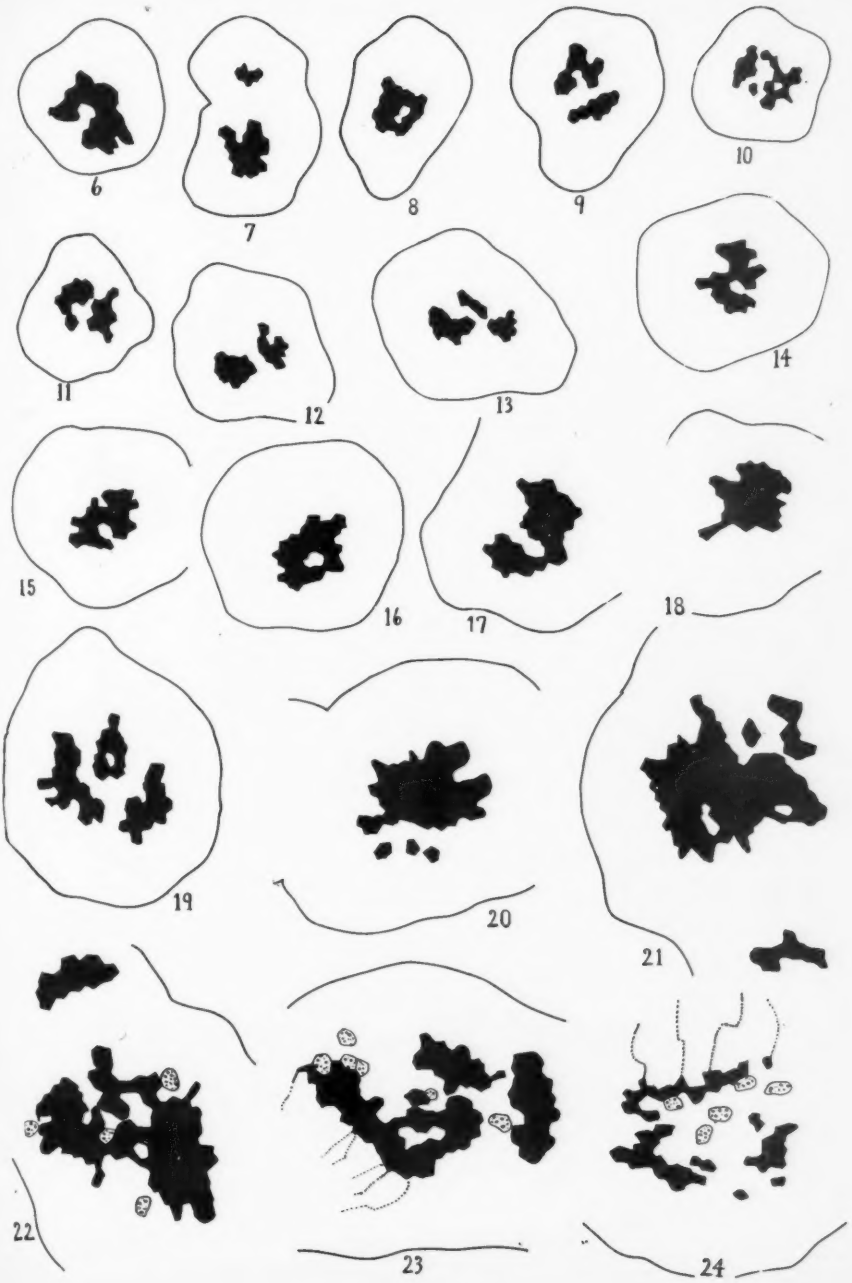

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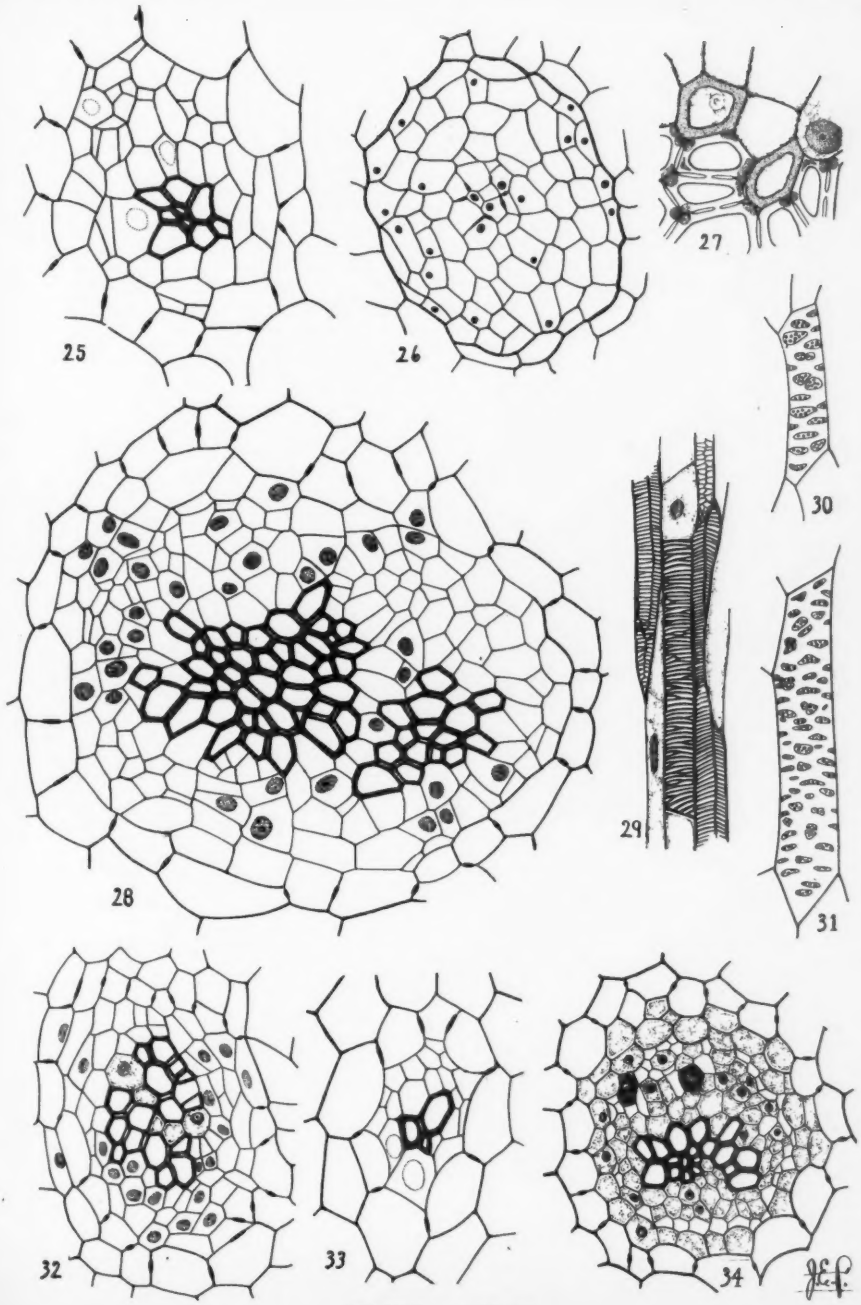

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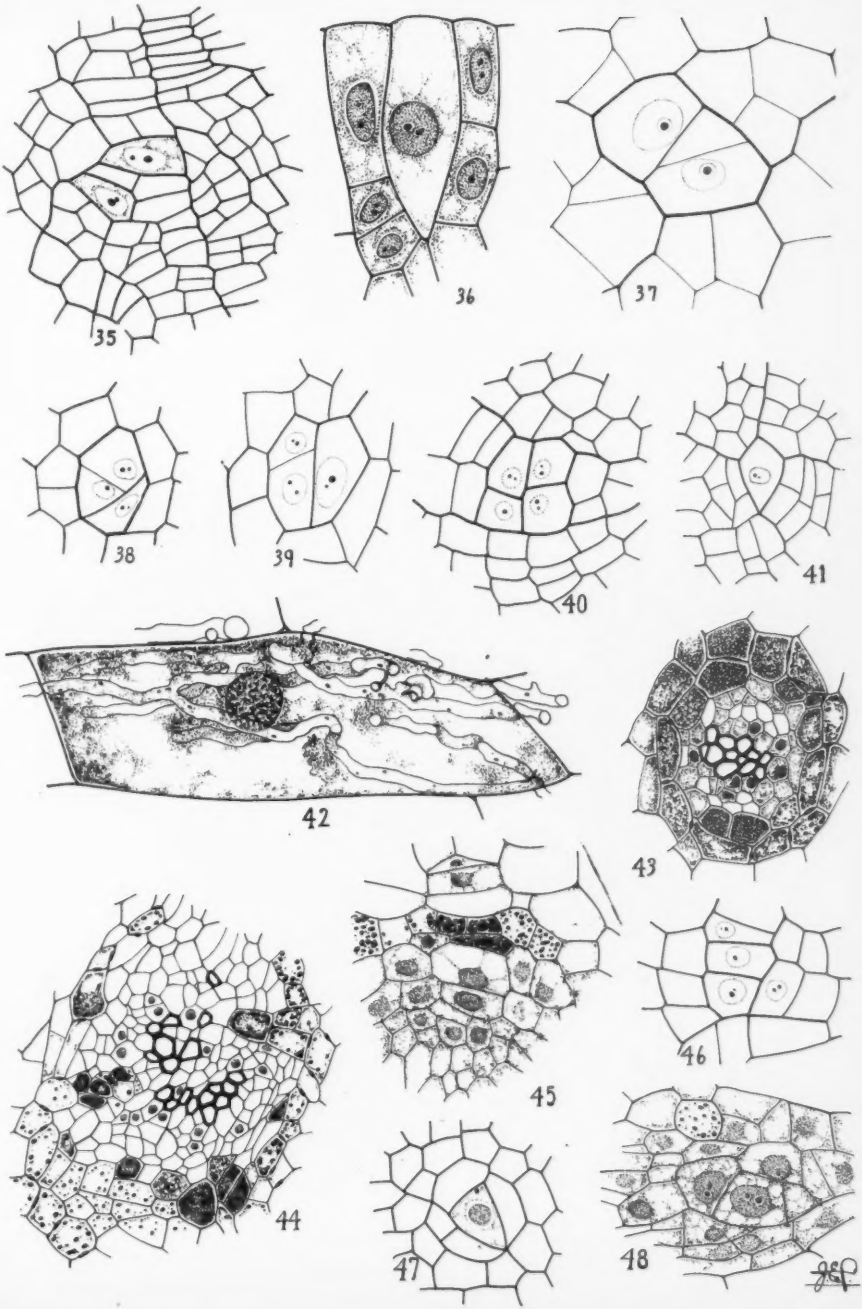

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SUBCORTICAL FORMATION AND ABNORMAL DEVELOPMENT OF STOMATA IN ETIOLATED SHOOTS OF OPUNTIA BLAKEANA

J. G. Brown

(WITH PLATES XXVII–XXX AND ONE FIGURE)

Introduction

Although experiments in the etiolation of plants date back to the activities of Charles Bonnet, their contributions to morphological botany have largely been incidental to the prosecution of physiological studies, and therefore extensive only as regards the number of species subjected to investigation. Even up to the present century, literature contains little more than observations on gross structural changes induced or accompanying etiolation, such as the elongation of internodes and peduncles, the dwarfing of leaves, and the underdevelopment of aerating and conducting tissues. To this literature it is believed that the material discussed in this paper adds several new and important facts. Since other investigators (**4**) have given reviews of the literature on etiolation, the writer will pass directly to his own studies.

Material

Opuntia Blakeana is a common, low-spreading, prickly-pear cactus on the mesas of southern Arizona, having joints 10 cm. in length, 9 cm. in breadth, and 1–2 cm. in thickness (fig. 1). The joints exhibit purplish areoles. Each areole near the margin and in the middle region of a joint bears one or two brown spines 1–3 cm. long. On the basal portion of a joint and on the lowest joints of the plant, areoles are often without spines. In addition to the spines, areoles frequently bear glochids about half the length of the spines, which are especially numerous near the apex of the joints. Both spines and glochids are of the usual barbed type found in cacti. Many bristles about 4 mm. in length, consisting of a single row of cells, are inserted near the bases of the

spines. Small awl-shaped leaves about 15 cm. in length are also borne just below the spines in the spring, but are soon abscissed. The plant grows in the most exposed situations. It was first described as a new species by ROSE (5) in 1909, and the plant from which the type specimen was obtained furnished most of the

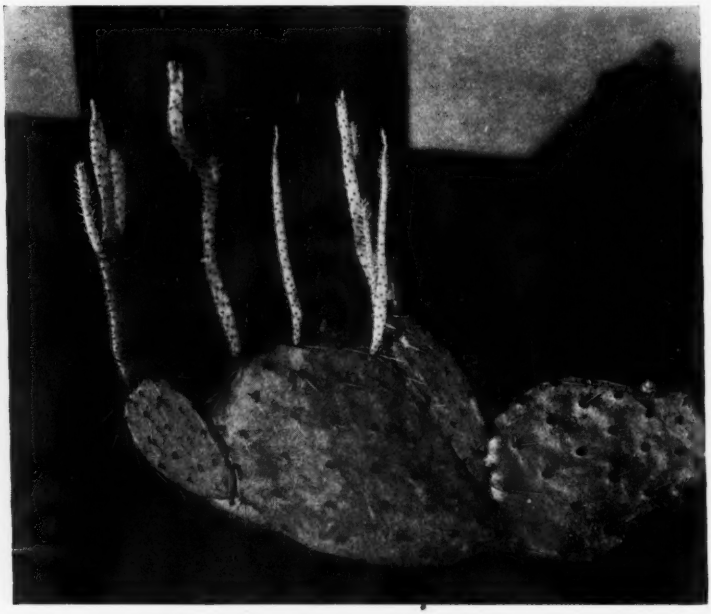

FIG. 1.—Etiolated shoots of *Opuntia Blakeana* sprouting from normal shoots which had been removed from open environment to dark chamber.—Photograph by D. T. MACDOUGAL.

normal shoots, and indirectly all of the etiolated shoots that were used in this study. The latter were grown from joints that had been removed to the dark constant temperature chamber of the Desert Laboratory.

Method

The chamber in which the etiolated shoots were grown has been fully described in publications of the Desert Laboratory,

but for convenience its main features will be given here. It is a small room situated under the floor of the main laboratory, from which light is absolutely excluded by means of an antechamber and two doors, the second door being a trapdoor in the ceiling of the dark chamber. The temperature remained between 56 and 75° F., with a maximum weekly fluctuation of 2° F. The humidity was 80–90 per cent. The room was absolutely dark at all times excepting for the candlelight used while collecting material.

Some of the etiolated shoots were killed in the dark chamber, others were removed to the light and allowed to grow for varying lengths of time before they were killed. The etiolated shoots removed to the light are referred to as etiolated-greened shoots. The killing agents used were chromo-acetic acid and Bensley's mercuric-formalin solution (1), and the material, both normal and etiolated, was then prepared for sectioning in the usual way. LAND'S (3) fixative proved useful in handling large sections. Several stains were used, including safranin-haematoxylon, orange G, and Haidenhain's iron-alum. Because of the mucilaginous nature of cactus material, it was found advantageous to continue the washing operation after the killing agent somewhat longer than is usually necessary with ordinary plant tissue.

Normal shoot

The gross structure of the normal shoot has already been given under the general description of the plant. Cross-sections (fig. 1) showed thick integumentary and palisade layers, an extensive spongy inner cortex, and a stele of elongated bundles. External to the phloem there appeared a mucilage mass which, with the bundle, presented a nail-shaped outline. The integumentary region consisted of cuticle, epidermis, and hypoderm (fig. 11). The cuticle averaged about 11 μ in thickness. Epidermal cells had straight anticlinal walls (fig. 4), and the longest cell measured was about 45 μ. One of the peculiarities of *Opuntia* and a few other cacti observed and mentioned by SCHLEIDEN (6) is the patchy decortication, in which the epidermal cells become active in spots, divide periclinally, and the inner of the two layers of cells thus formed continues to divide until a layer of tissue several

cells thick is present, whose outermost wall is the former external wall of the old epidermis. The tissue developed in this way is eventually entirely cut off, and a new outer epidermal wall is cutinized, so that the new epidermis resembles that which has just been lost.

The stomata were of the regular dicotyl type (fig. 4) and numbered 32–36 per sq. mm. of surface. Because of the thick cuticle they were sunken below the surface of the shoot (fig. 14). Below the epidermis extended the hypoderm, about six cells in thickness (figs. 11, 14). The first layer of the hypoderm consisted of a sheet one cell thick, with a large crystal of calcium oxalate in almost every cell. The remainder of the hypoderm was made up of about five layers of stone-cork cells. In places the crystal-containing cells almost closed the air chambers just under the stomata. The stone-cork cells were deeply pitted. They gave place abruptly to the palisade tissue with its long tubelike columns of cells, which was followed by the spongy cortical region reaching to the stele. The most external chloroplasts occurred in the palisade cells, where they were numerous on the lateral and end walls. Large air chambers extended from the stomata inward through the hypoderm into the palisade tissue, and the intercellular spaces were extensive in both palisade and spongy inner cortex. The structure of the stele will be described in a future paper.

Etiolated shoot

The appearance of the etiolated shoots presented a marked contrast with that of the normal ones (fig. 1). They were pinkish at first, but later changed to a very light green. They were longer than the normal shoots and more or less flattened in cross-section (fig. 3). Numerous sessile leaves like those of the normal plant in form were produced, which persisted for a short time. The longest measured was 7.5 mm. Spines, bristles, and glochids were grouped in a normal manner but were reduced in size, the spines averaging 3 mm. in length, the bristles about 1.5 mm., and the glochids intermediate between the two.

Unlike that of the normal plant, the epidermis of the etiolated shoot was without cuticle (fig. 13), although the walls showed

more or less cutinization. Individual cells varied in form and size from the base to the apex of the shoot. Those in the apical region were much elongated (fig. 6), some of them measuring 175 μ. Their lateral walls were straight, and their shorter end walls were wavy in outline. In the basal region the epidermal cells were not longer than 85 μ, and nearly all of the walls were slightly wavy when seen from the surface of the shoot (fig. 5).

However striking the changes in external form and gross structure due to etiolation may appear, the internal changes were even more interesting. When the surface of the etiolated shoot was examined under low magnification, numerous small elevations were observed which reflected the light. Closer investigation showed these structures to be minute papillae, each one bearing a stoma at the apex (figs. 13, 25, 27). Their nature was better seen in longitudinal and transverse sections of the shoot (figs. 25, 26), and they were usually found to be epidermal, the entire structure arising in most cases from a single cell; in others, chiefly from a single cell, but augmented to some extent by division of neighboring cells of the cortex. The papillary initial appeared to be analogous to a stoma mother cell. Evidently the stimulus to division had continued to act on the stoma mother cell and its progeny for some time and had met with a response, even after cutinization of the surface cell walls had occurred, for in one instance the resulting structure, unable to grow outward, had pushed inward among the undifferentiated cells of the cortex (fig. 28). The first division of the papillary initial was either a vertical one, as in the ordinary process of stoma formation (figs. 7–9), or else the cell became papillate and divided by an oblique wall (figs. 18, 19). The lower of the two cells thus formed then divided by a vertical wall, and the upper cell followed with a transverse division (fig. 20). After this the walls were chiefly oblique. Eventually two guard cells were cut off, which, seen from the surface, resembled the guard cells of a normal stoma (fig. 27), but which were more or less wedge-shaped in transverse sections of the shoot (figs. 21–25). The mature papilla might be compared to a hydathode in surface view, but in internal structure and in origin it was very different; the tissue was not glandular, no

vascular or conductive elements of any kind ended near it, and it usually originated from a single epidermal cell. Although large and solid papillate structures were most numerous, every transition was found between that type and the simplest form of stoma. Thus some papillae consisted of only a few cells in addition to the stomatal guard cells (fig. 26), and there was every gradation in size from this up to a papilla of dozens of cells. Some of the papillae were hollow also, and it appeared that the cavity had formed by the breaking down of the internal cells into a mass resembling mucilage. The simplest stomata consisted of guard cells with no differentiated auxiliary cells (figs. 32, 33), and often with neither intercellular space nor air chamber below. A few normal stomata had mucilage masses just underneath the stomatal opening.

Another peculiar situation was suggested by the occasional occurrence of a perfectly developed stoma lying almost under the margin of decorticating patches, and it was finally disclosed after a careful search. Three stomata were found developing under several layers of cork cells, one of which had guard cells just beginning to split apart (fig. 30). So far as can be discovered, the subcortical formation of stomata has never been reported. Perhaps it does not occur outside of the cacti.

In numbers the stomata of the etiolated shoot ran far below the normal plant, 12–16 being the maximal numbers found per sq. mm., even in the lower part of the shoot where they would be expected to be most numerous, in agreement with the nature of the epidermis which here most closely resembled that of the normal shoot. The stomata were mostly open.

The regions of a cross-section of the etiolated shoot also contrasted sharply with those of the normal stem (figs. 1, 3, 11, 13, 17). That the cuticle was absent has already been mentioned. The epidermis consisted of cells much broader than normal and much better supplied with protoplasm. Large budding chloroplasts were present which resembled chains of yeast cells in form, and varied in size from 30 μ to granules too small to be studied with the highest available power of the microscope (fig. 17). Below the epidermis there was neither a crystal-containing layer

nor stone-cork hypoderm, nor was a palisade tissue differentiated, but two general physiological regions could be recognized: an outer leucoplast-containing one 3–5 layers of cells thick, and an inner starch-containing one reaching to the stele (fig. 13). The leucoplasts of the outer cortical region were actively budding. like the chloroplasts of the epidermis, but were mostly smaller, and the smallest sizes increased in number in proportion to the distance from the surface of the shoot. Air spaces were much less extensive than in the normal cortex, and were often formed abnormally, as previously described.

Etiolated-greened shoot

Etiolated shoots placed in the laboratory windows and those transferred to the open presented similar changes in structure, but the changes were more rapid in the latter environment. Decortication removed the abnormal stomata with the papillae, and none reappeared (figs. 15, 29). Chloroplasts quickly disappeared from the epidermal cells. The whole shoot presented a shrunken appearance, due not only directly to the water loss from the almost unprotected tissues, but also to the actual death of many of the cells in the outer cortex (figs. 15, 16), a process by which air cavities were quickly enlarged. As cutinization progressed in the epidermis, and the turgidity of the cortical cells gradually became restored, the whole topography of the cross-section changed, for palisade tissue had appeared (fig. 12). Intracellular changes also occurred in the cortical cells. The chloroplasts were reduced in number as compared with the leucoplasts in the outer cortex of the etiolated shoot. They were regularly rounded in form and were present in cells as deep as the stelar region (fig. 12). They were necessarily confined to the peripheral region of the cell because of extensive vacuolization, and were found on end walls and lateral walls.

The new branches that appeared from the buds that had formed on the etiolated shoots before their removal to the light were larger, both in breadth and thickness, than the branches of the etiolated stems; and spines, glochids, and bristles were more like those of the normal plant in size and general appearance.

Discussion

The preceding description of the results obtained in the etiolation of *Opuntia Blakeana* suggest for discussion the factors concerned in the development of cuticle; the outline of epidermal cells; the number, origin, and development of stomata; the formation of palisade tissue; and the appearance of air spaces.

Cuticle formation, it has been suggested, approaches a maximum when transpiration is great in amount, and when it is high in proportion to absorption (**2**). It has also been stated that cuticle formation is favored by growth in concentrated nutrient media. Both factors were probably operating in these experiments. The etiolated shoots certainly transpired much less than they would have done had they been grown in the open, for they promptly wilted when transplanted to the latter environment. Gradual increase in the osmotic pressure of the sap in the joints from which the etiolated shoots obtained their nutrient supply must have occurred, but it was evidently insufficient to induce the development of cuticle. MACDOUGAL found no cuticle formation in any of the numerous plants with which he experimented.

The changes mentioned in connection with the outline of the epidermal cells have been observed by many investigators. Whether light is a factor in determining the shape of the cell walls in the epidermis could not be determined, for an attempt was not made to reduce transpiration when etiolated shoots were removed to an outdoor environment in order to separate the two factors. Mesophytic conditions in the dark chamber may be considered favorable to crenated walls in view of the results of other workers (**2**).

Decrease in the number of stomata per unit of surface area is in general harmony with the results of numerous investigators. Six or seven times as many epidermal cells per unit of surface appeared on the normal shoots, while the stomata were two to three times as numerous, compared with the etiolated ones. In his experiments with *Opuntia Opuntia*, MACDOUGAL found that the stomata on the etiolated shoots were reduced in size. Those measured in *Opuntia Blakeana* were not different in size from the stomata of the normal plant, excepting a few freakish forms of stomata.

Development of stomata under cortical tissue is a phenomenon that is difficult to explain. COWLES states that although the factors inducing the appearance of stomata are unknown, evidence is not lacking that light favors their development, and that stomata are abundant where transpiration is vigorous, and absent where it is reduced or wanting. Neither factor could have greatly influenced the development of subcortical stomata in a direct way in this case, for light was absolutely excluded and transpiration was much reduced, owing to the increased moisture content of the air in the dark chamber and the covering of cortical cells. In connection with the light factor, however, there is another interesting possibility. In MACDOUGAL's experiments, seedlings of *Aesculus*, whose basal internodes were briefly illuminated, developed laminar bodies in internodes formed some weeks after the stimulus had been given, which were entirely lacking in the absolutely etiolated seedlings. He states that "the stimulative effect of illumination . . . . may be received by one portion of the body and transmitted to another, and the impulses may even be communicated to organs not actually formed at the time the stimulating rays were received." The last resort appears to be to ascribe the phenomenon to some internal factor such as is included under the term heredity.

The papillary structures appear to be peculiar to the etiolated shoots of *Opuntia Blakeana*. That they result from the division of a cell analogous to a stoma initial seems to be a logical conclusion, since all stages are found, from the normally developed stoma with two guard cells and two adjacent cells originating from one initial, to the elevated structure consisting of many cells around and below the guard cells, all developed from one initial. The fact that the first division may be a periclinal one appears to be a matter of detail that does not exclude such an interpretation. Some stimulus, possibly previous illumination or some internal stimulus, starts the division process, which is favored by the increased moisture of the dark chamber and by an abundant food supply from the normal shoots at the base of the etiolated ones. Once started, the divisions continue until the cutinization of the epidermal walls offers sufficient resistance to check the growth

of the papilla. The appearance of the abnormal structure shown in fig. 28 seems to favor such a conclusion.

Among the theories advanced to explain palisade development are the light theory, the transpiration theory, and the lateral pressure theory (2). No attempt was made to separate the light and transpiration factors when the etiolated shoots were transplanted. Both factors were increased upon transferring the etiolated shoots to the light. Lateral pressure must have been reduced, for the cortical tissues had a minimum of intercellular air spaces in the dark chamber and a maximum in the etiolated-greened condition. Furthermore, the collapse and death of many cortical cells after exposure of the shoots to an outdoor environment must have further reduced lateral pressure. Another factor may have been operative. The partially cutinized epidermal walls resisted collapse, judging by the appearance of sections of fresh and killed tissues, and this cylinder of epidermis, as it gradually decreased its water loss by increased cutin secretion, must have set up an outwardly directed strain that could not fail to influence the shape of the cells attached to it.

The appearance of large intercellular spaces in the outer cortex is closely related to palisade development which has already been briefly discussed. Shrinking and subsequent turgidity and the death of cortical cells were important factors in increasing the intercellular spaces and enlarging air chambers. All of these factors are probably active in various plants growing in a natural environment in the southwest. It has already been shown for succulents that shrinkage and expansion are marked with the change from wet to dry seasons (7). An investigation of plants that survive continued dry winds would probably reveal intercellular changes in the cortex similar to those observed in these experiments in the etiolated-greened shoots.

Summary

1. This paper deals with a comparison of the appearance, form, and structure of normal, etiolated, and etiolated-greened shoots of *Opuntia Blakeana*, a prickly-pear cactus of the southwest.

2. The flat spiny joints of the normal plant, when carried into the dark chamber, produced roundish, light green, elongated, etiolated shoots which differed remarkably in form and structure from those of the normal plant, and which exhibited structural changes when transplanted in an outdoor environment that brought them to resemble the normal shoot.

3. The etiolated shoots lacked a cuticle, developed papillate structures and stomata abnormal in form and position, and lacked the cortical differentiation so characteristic of the normal shoot.

4. The etiolated-greened shoots lost water rapidly at first. Their air spaces increased rapidly by active intercellular splitting of walls and by the collapse and death of cells; then a cuticle appeared; cortical cells elongated, forming palisade tissue; and in other respects the shoots approached the normal ones in structure.

The study presented in part in this paper was made possible through the interest of Dr. D. T. MacDougal, Director of the Desert Laboratory, who furnished the etiolated material used, and whose friendly criticism constituted a continual source of inspiration. Acknowledgments are also due Professors John M. Coulter, Charles J. Chamberlain, and W. J. G. Land, of the University of Chicago, for numerous helpful suggestions.

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EXPLANATION OF PLATES XXVII–XXX

PLATE XXVII

FIG. 1.—Cross-section of normal shoot showing topography: *a*, integumentary region; *b*, palisade region; *c*, mucilage mass at peripheral side of vascular bundle; *d*, stelar region; ×0.5.

FIG. 2.—Cross-section of etiolated-greened shoot showing topography: *a*, epidermis; *b*, cortex; *c*, leaf trace; *d*, vascular bundle; *e*, stele; ×12.

FIG. 3.—Cross-section of etiolated shoot showing topography; same ettering as fig. 2; ×12.

FIG. 4.—Epidermis from normal shoot after removal of cuticle; ×395.

FIG. 5.—Epidermis from basal region of etiolated shoot; ×395.

FIG. 6.—Epidermis from apical region of etiolated shoot; papillae not yet fully developed; ×395.

FIGS. 7–10.—Normal development of stoma in epidermis of etiolated shoot; fig. 8 is vertical section of stage shown in fig. 7; ×525.

PLATE XXVIII

FIG. 11.—Part of transverse section through outer region of normal shoot: *a*, epidermis with layer of cuticle above; *b*, hypoderm; *c*, palisade region; first layer of hypoderm is a crystal-containing sheet; 2 air chambers shown; ×85.

FIG. 12.—Part of transverse section through etiolated-greened shoot reaching from epidermis into stele; palisade tissue has appeared; round bodies represent chloroplasts; *a* and *c* as in fig. 11; *d*, inner spongy cortex; ×85.

FIG. 13.—Part of transverse section of etiolated shoot reaching from epidermis into stele; papilla shown in upper right-hand corner and part of mucilage mass in lower left-hand corner; black dots represent leucoplasts, and circles represent starch grains; ×85.

FIG. 14.—Detail of integumentary region of normal shoot; lettering as in fig. 11; ×525.

FIG. 15.—Part of transverse section of etiolated shoot shortly after removal to light; decortication of outer layers of shoot involving a stoma in progress; *a*, future surface wall of new epidermis; ×525.

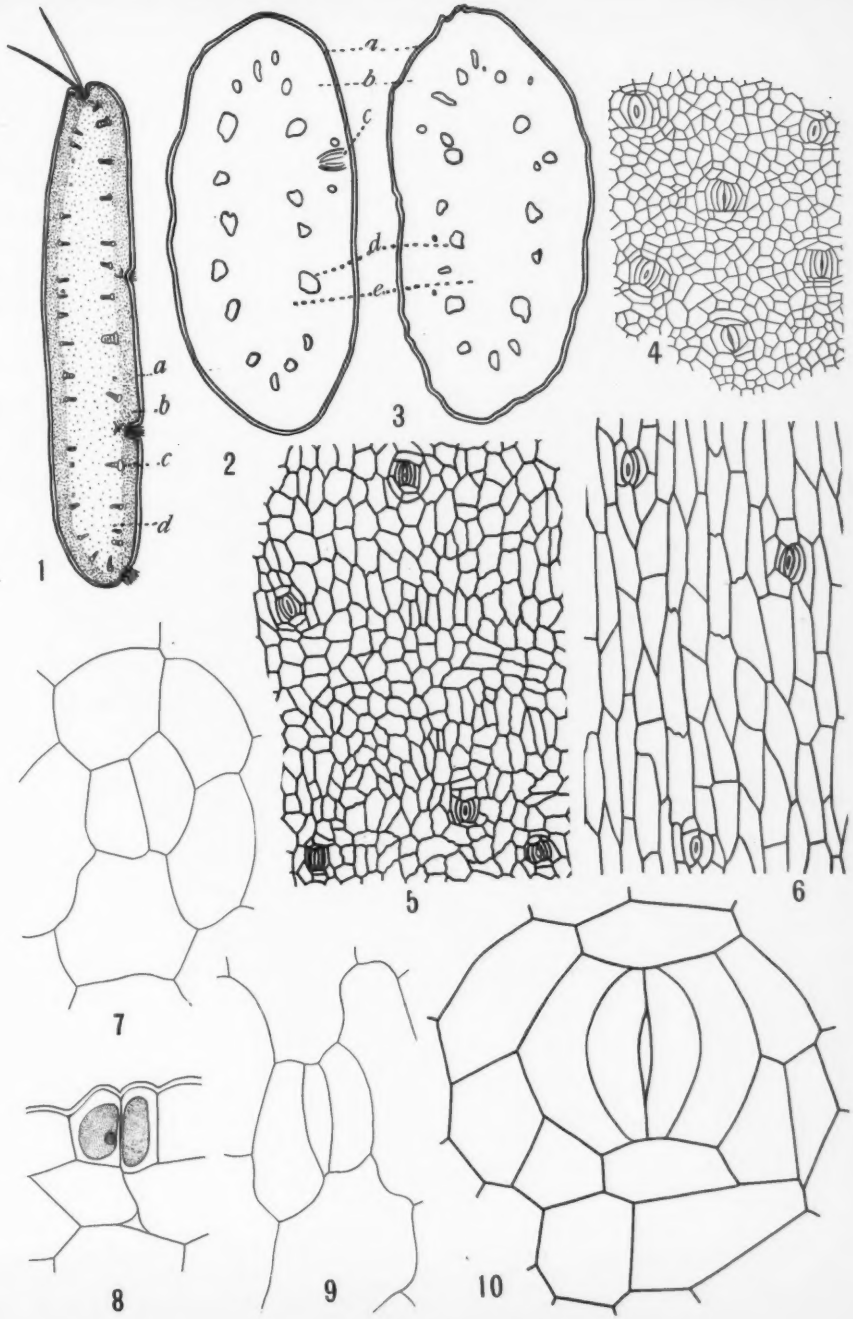
FIG. 16.—Detail of stage of adjustment of etiolated-greened shoot shown in fig. 12; remains of dead cell showing below, projecting into air space; ×525.

FIG. 17.—Detail of transverse section shown in fig. 13; reticulate bodies are nuclei; irregular bodies are chloroplasts and leucoplasts; large, round, lightly stippled bodies are grains of storage starch; ×525.

PLATE XXIX

FIGS. 18–25.—Development of papilla as seen in transverse sections of etiolated shoot: *a*, inner wall of initial cell; *b*, first transverse wall; *c*, stoma; fig. 19, ×390; figs. 20, 22, ×245; all others, ×525.

FIG. 26.—Vertical section of young papilla cut parallel with long axis of etiolated shoot, showing side view of guard cell; ×525.



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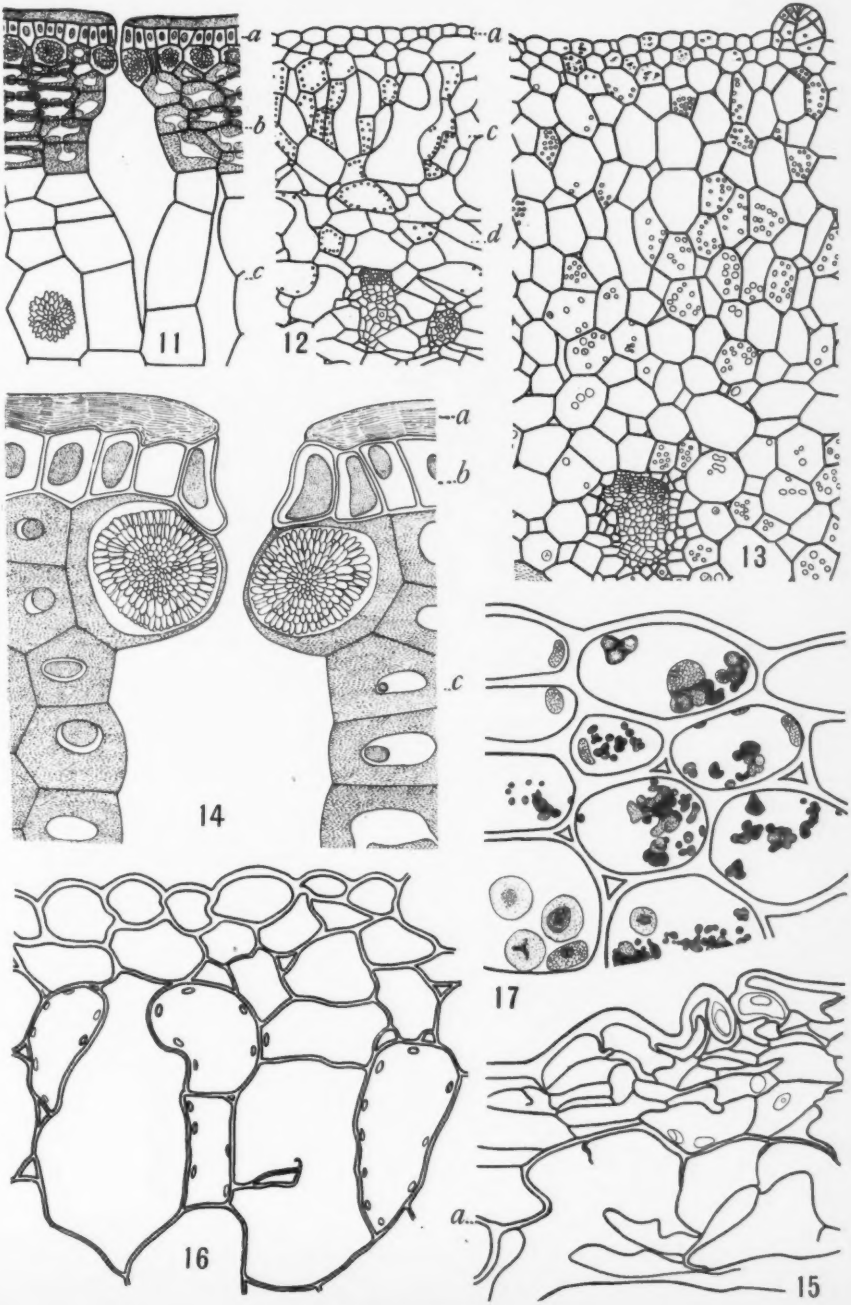

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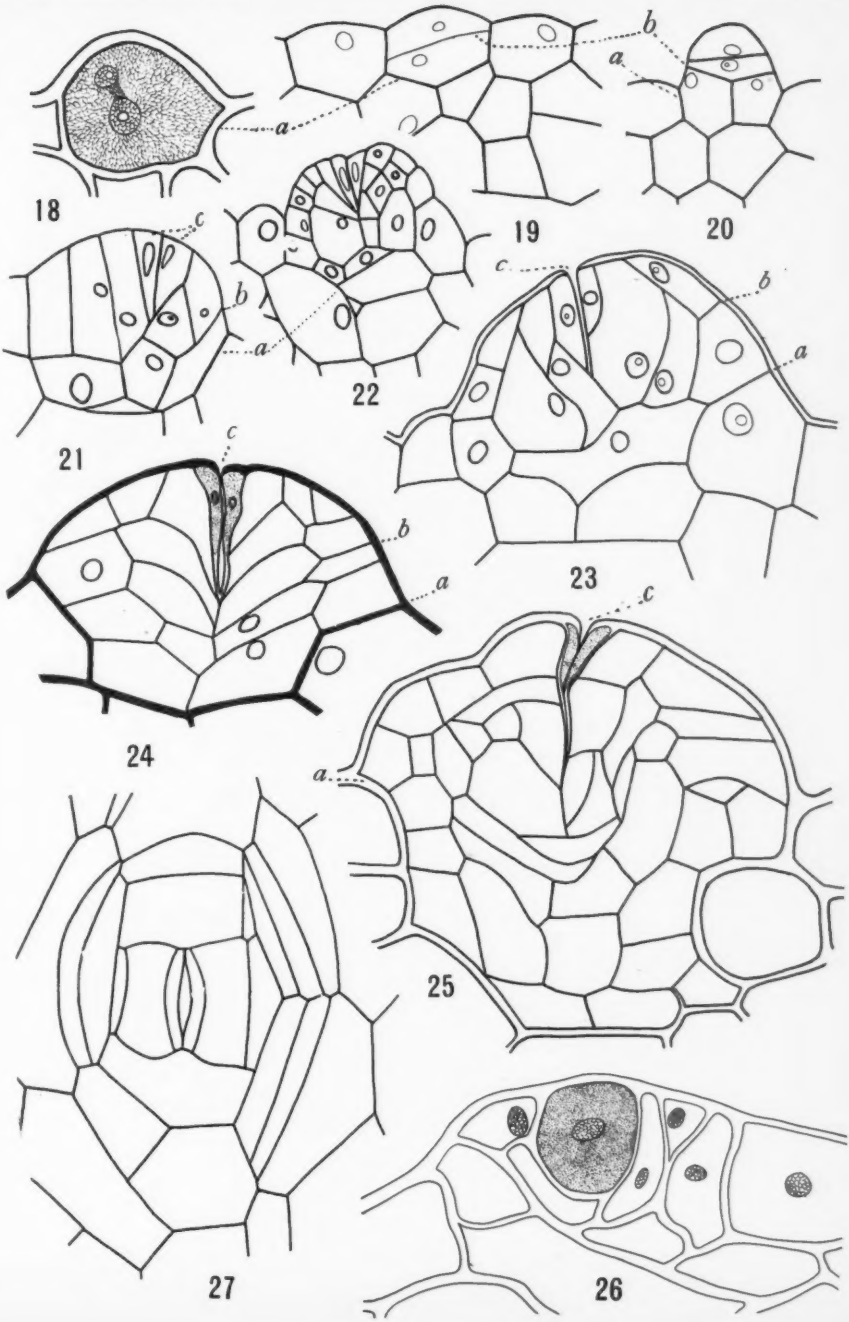

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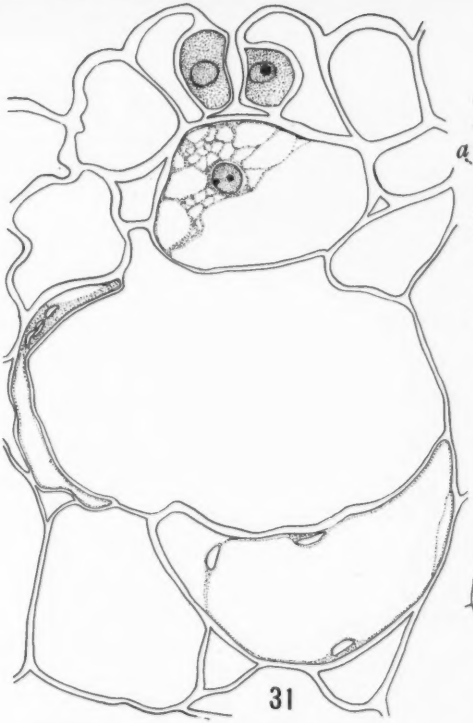

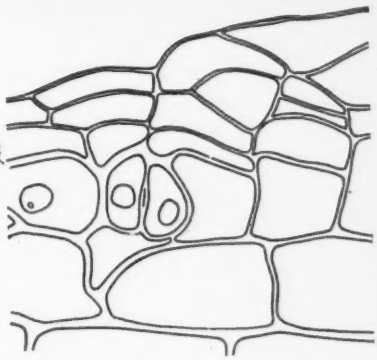

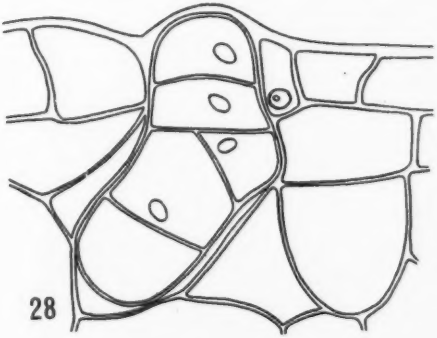

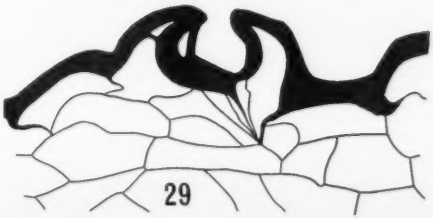

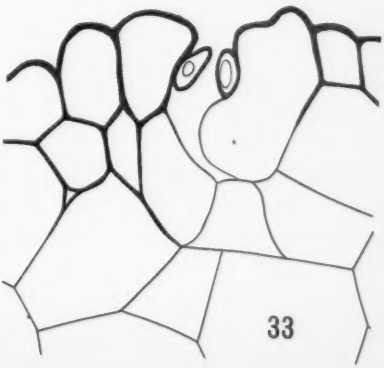

BROWN on OPUNTIA

PLATE XXX

FIG. 27.—Surface view of papilla with central stoma; ×525.

FIG. 28.—Structure found in cross-section of etiolated shoot arising like papilla, from epidermis, but growing inward; ×525.

FIG. 29.—Mass of decorticated tissue including papilla, drawn from transverse section of etiolated-greened shoot; ×525.

FIG. 30.—Subcortical development of stoma drawn from transverse section of etiolated shoot: *a*, future external epidermal wall; ×525.

FIG. 31.—Part of transverse section of etiolated-greened shoot showing stoma not yet connected with air chamber; collapsed cortical cell also appears to left; ×525.

FIG. 32.—Part of transverse section through etiolated-greened shoot: stoma lacked auxiliary cells and opened into air chamber of considerable size; similar simple stomata present in etiolated shoot; ×525.

FIG. 33.—Freakish stoma found in transverse section of etiolated shoot; ×395.

COMPOSITION OF GASES IN INTERCELLULAR SPACES OF APPLES AND POTATOES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 271

J. R. MAGNESS

(WITH ONE FIGURE)

Introduction

During a study of the ripening processes in fruits, and the chemical and physiological changes associated with them, the question has arisen as to what may be the composition of the gas in the intercellular spaces. The gas within the tissues constitutes in part the medium in which the processes associated with the life of an organism take place. It is only reasonable to suppose that the composition of this medium may exert some influence upon the rate or nature of the changes taking place. The difficulty of extracting the gases from the interior of the tissues is probably responsible for the fact that plant physiologists have almost entirely neglected studies along this line. GERBER (5) reports work of FREMY published in 1840 and 1860, in which the gas contained in apples was analyzed at intervals during their development and ripening. He found oxygen more abundant in the green fruit, the amount decreasing as the fruit matured on the tree. We have, however, no critical studies upon the internal gases of plant tissues.

An apparatus has been devised for obtaining a sample of the gas from within the tissues, without contamination with air. It is the purpose of this preliminary report to describe the apparatus and methods of sampling, together with the data secured, in order that they may be available to workers along related lines.

Apparatus

The apparatus used in extracting the gas is shown in fig. 1. It consists of a leveling bottle or burette (*A*), connected through heavy walled rubber tubing to a side neck at the base of a thick-walled glass cylinder (*B*). This cylinder is flared at the top, and

fitted with a ground glass stopper (*C*) in which is sealed a capillary tube. The flare in the top of the cylinder should be sufficient to allow the stopper to set well down (as illustrated), in order to permit a mercury seal above the stopper. The capillary tubing is bent around in the manner shown, in such a way that the tip can be immersed in a vessel of mercury (*D*). Glass stopcocks (*E* and *F*) must be in the positions shown. It is especially important that the cock *F* be as indicated, rather than directly above the cylinder, for by the former arrangement any small leak about the cock can quickly be detected. Cocks and stopper should be kept well

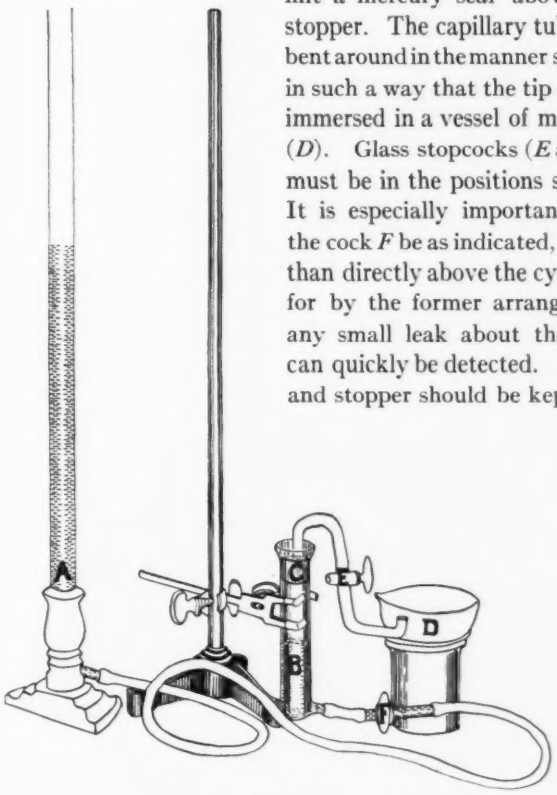

FIG. 1

coated with heavy desiccator grease. The proper dimensions for the cylinder (*B*) will obviously vary with the type of tissue being examined, and the volume of this tissue necessary to secure a gas sample adequate for an analysis. For work with apple and potato tissue, a cylinder 6 inches long and 1 inch inside diameter has been found very satisfactory.

Method

By opening the stopcock *E* and *F*, mercury was allowed to flow from the leveling burette into the cylinder until the latter was about two-thirds full. A plug of the tissue from which the gas was to be extracted was then cut with a cork borer. By using a sharp borer and cutting clean, the epidermal layers sealed each end of the plug of tissue, while all cut surfaces were in close contact with the walls of the cork borer. Consequently, there was no opportunity for contamination of the sample with the air.

The tip of the cork borer was then put under the mercury in the cylinder, and the sample plug of tissue pushed out under the mercury; a long glass rod has been found satisfactory for this. The plug was held beneath the mercury while the rod was replaced by a wire spring, and the ground glass stopper fitted into the cylinder. A mercury seal above the stopper precluded the possibility of leaks. The stopcocks (*E* and *F*) were again opened, and all air in the cylinder and capillary tubing replaced by mercury. The tip of the capillary was immersed under mercury in *D*, the cock (*E*) closed, and the leveling burette (*A*) lowered. The partial or almost complete vacuum in the cylinder causes the gas in the tissue to expand; it escapes from the tissue and collects in the top of the cylinder. The first few bubbles of gas were always discarded by driving them out through the capillary. When sufficient gas for the final analysis had collected in the top of the cylinder, it was driven off and collected over mercury in a small vial. The sample was transferred to a Bonnier-Mangin gas analysis apparatus of the type described originally by AUBERT (**2**) and later by GRAFE (**4**). CO_2 absorption was by means of 15 per cent KOH; oxygen was absorbed by 8 per cent pyrogallic acid in 30 per cent KOH. About one-half cc. of gas is sufficient for an analysis in this apparatus.

Results

Three boxes of Yellow Newton apples, representing three different trees at Watsonville, California, were used in the analyses of gas in apples. Some apples from each box were stored in a refrigerator at 6° C. and at 11° C. Others were stored in a vessel immersed in a water bath held at 20° C.; while a fourth lot was

held in an oven at 30° C. A few were held also at 2° C. In all cases abundant aeration was provided to prevent the possibility of an accumulation of CO_2 in the air surrounding the fruit. A summary of the data on the internal atmospheres in apples is given in table I.

TABLE I

ANALYSES OF GAS IN INTERCELLULAR SPACES OF YELLOW NEWTON APPLES

Temperature of storage °C.	Number of determinations	Percentage CO_2	Percentage O_2	Percentage CO_2+O_2	Percentage N_2 by difference
2.....	5	6.7	14.2	20.9	79.1
6.....	30	8.4	12.9	21.3	78.7
11.....	27	12.2	10.7	22.9	77.1
20.....	31	17.2	5.5	22.7	77.3
30.....	29	21.4	3.2	24.6	75.4

The data presented in table I require but little discussion. It is apparent that the percentage of CO_2 in the gas within the tissues increases markedly at the higher temperatures. At the same time there is a corresponding decrease in the percentage of oxygen present, the average ranging from 14.2 per cent at 2° C. to only 3.2 per cent at 30° C. These data, representing averages of a number of determinations, clearly indicate the marked variation that may occur in the composition of gas in the tissues under varying conditions of temperature.

It is of interest to note that at the lower temperatures the total percentage of oxygen plus that of carbon dioxide is about equal to that of the air. At the higher temperatures, however, and in association with the decreasing amounts of oxygen in the tissues, the sum of these two gases gradually increases. This would indicate that at the higher temperatures one molecule of oxygen liberates more than one molecule of CO_2. Such data accord with the work of GERBER (5), who found that in fleshy fruits stored at high temperatures acids were mainly respired, and that the ratio of CO_2 to O_2 under these conditions was considerably superior to unity. There is also the possibility that at the higher temperatures a certain amount of anaerobic respiration is going on, due to the relatively small amount of oxygen present. This would result in

an increased amount of CO_2 in the tissues, without a corresponding decrease in oxygen.

Table II gives the data obtained for potatoes. The potatoes used were purchased on the open market, and the variety was not determined. They were sound, smooth, and of uniform average size. A few carrots were also studied, for comparison with the apples and potatoes. From the data presented it is apparent that the same general tendency holds in potatoes and carrots that was noted in apples, that is, an increasing percentage of CO_2 and a decreasing oxygen content at higher temperatures.

TABLE II

ANALYSES OF GAS IN INTERCELLULAR SPACES OF POTATOES AND CARROTS

Temperature of storage °C.	Number of determinations	Percentage CO_2	Percentage O_2	Percentage CO_2+O_2	Percentage N_2 by difference
	Potatoes				
11	8	19.6	10.9	30.5	69.5
22	8	34.4	5.7	40.1	59.9
	Carrots				
11	2	12.2	13.1	25.3	76.7
24	2	28.6	5.2	33.8	66.2

It will be noted that the total CO_2 and oxygen is much higher in the case of potatoes than was found in apples. This variation may be due in part to the fact that there is relatively much less intercellular space in potatoes than in apples, and a higher percentage of the gas may have come out of solution in the juice in the samples obtained from potatoes than in those obtained from apples. It is necessary to use much larger samples of potato tissue in order to obtain sufficient gas for an analysis than is essential when apple tissue is used.

The amount of gas that may be coming out of solution in the juice, rather than from the intercellular spaces, presents a difficulty inherent in this method of sampling. There is no assurance that the gas that comes out of solution is of exactly the same composition as that of the intercellular spaces. The consistent results recorded

for fruit under the different temperatures tested, however, clearly indicate the tendency of the oxygen-carbon dioxide ratio within the tissues, regardless of the fact that the absolute values may vary somewhat.

Effect of wounding

Many references to the effect of wounding plant tissues upon rate of respiration are found in the literature. Invariably wounding of the tissue has resulted in an increased rate of respiration. GERBER has found this to be true of apples, grapes, and other fruits. APPLEMAN (1) has reported the same phenomenon for white potatoes.

A few apples were prepared for a study of the effect of wounding upon the composition of the internal atmosphere. A thin slice of the peel was removed from each end of the fruits, and they were then put in storage at the various temperatures by the side of whole fruits serving as checks. The data from the analyses of these fruits are reported in table III. It is apparent from these data that removing the epidermis greatly facilitates the entrance of oxygen to the tissues, and also the escape of accumulated CO_2. It would be interesting to know to what extent increased respiration following wounding is due to mechanically facilitating this gaseous exchange, and to what extent it is due to actual metabolic changes in the wounded tissues.

TABLE III

EFFECT OF REMOVING PEEL FROM ENDS OF FRUITS UPON COMPOSITION OF INTERNAL ATMOSPHERE

Temperature °C.	Treatment	Number of Determinations	Percentage CO_2	Percentage O_2	Percentage CO_2 + O_2
1.....	Whole apples	3	6.6	14.6	21.2
1.....	Ends peeled	4	1.7	15.8	17.5
20.....	Whole apples	2	17.8	7.0	24.8
20.....	Ends peeled	2	7.4	9.9	17.3
30.....	Whole apples	2	23.9	1.8	25.7
30.....	Ends peeled	2	12.6	8.9	21.5

Variation in composition of gases

Considerable variation occurred between individual apples or potatoes held under identical conditions. This is to be expected

when the wide variation in size, thickness of epidermis, etc., is considered. The extremes of variation found in apples held at 20° C. are indicative of the range of fluctuation that may be encountered in work of this type. The 31 apples analyzed at this temperature contained gas averaging 17.2 per cent CO_2. The minimum CO_2 recorded for any apple of the lot was 12.5 per cent; the maximum 25.7 per cent. Only one apple, however, showed more than 21.8 per cent, so that this latter figure is a more accurate maximum. The extremes of oxygen variation were somewhat less. With an average of 5.5 per cent oxygen, the minimum value was 1.0 per cent, and the maximum 9.5 per cent. Although most of the values were very much nearer the mean than these, it is essential that a considerable number of individual analyses be made to determine the true mean for any given condition.

Factors influencing amount

Three main factors operate to determine the amounts of CO_2 and oxygen in the intercellular spaces at any given temperature. These are (1) the rate of oxidation, or the rate at which oxygen is taken up from and CO_2 given off into the intercellular spaces; (2) the permeability of the skin or epidermal covering to CO_2 and oxygen; and (3) the difference in pressure of CO_2 and oxygen within and without the fruit, which determines the rate of gaseous exchange when the permeability factor is constant. The effect on each of these factors of varying the temperature will explain the variation occurring in the internal atmosphere of the tissues studied at the different temperatures.

EFFECT OF TEMPERATURE ON OXIDATION PROCESSES.—GORE (**6**) has found that the rate of respiration for a large number of fruits, as measured by the quantity of CO_2 given off, increased, on an average, 2.38 times for a 10° rise in temperature. Enzymatic processes in general, within the range of temperatures here studied, show an increase of from two to three times for each 10° rise. It is thus apparent that the oxidative processes will be speeded up very markedly by temperature increases.

EFFECT OF TEMPERATURE ON PERMEABILITY.—DENNY (**3**), in a study of the permeability of a number of plant membranes, has

found that in general the increase in permeability per 10° rise in temperature varies from 1.3 to 1.8 times, averaging about 1.5. These data are based on permeability to water, but there is no reason for believing that gases would be fundamentally different. The diffusion of gases in all probability is mainly a physical process, and as such is relatively much less affected by temperature changes than the chemical changes involved in oxidation.

From a consideration of these relative effects of temperature on oxidation and on permeability, it is apparent that the absorption of oxygen and release of CO_2 are increased much more by a given rise in temperature than is the tendency for oxygen to be supplied to the tissues, and CO_2 to be given off from them. Consequently, as the temperature is raised, the amount of oxygen in the tissues becomes less and less, while the CO_2 accumulates correspondingly. This continues until the third factor becomes effective, that is, the difference of CO_2 and oxygen pressures within and without the fruit becomes so great that equilibrium is again established.

Significance of ratio

No attempt has been made in this preliminary work to associate the percentages of CO_2 and oxygen found with the processes taking place in the fruit. The data presented, however, clearly indicate the necessity of taking this factor into consideration in many types of horticultural and physiological investigations. It should be given attention in studies of the effect of temperature upon the processes in plant tissues, for it is readily apparent that much variation may be caused by the composition of the medium in which these processes are carried on. Of special importance is the application of studies of this type to the questions as to the effect of wounding and various other treatments on the respiratory processes in tissues. Finally, it is of prime importance to know the composition of the internal atmosphere in studying the effects of various gases, etc., on plant organs. Some work has been done on the effect of various gases on fruits and vegetables in storage. Obviously it is essential in such work that the composition of the internal atmosphere be known.

The writer feels deeply indebted to Dr. WILLIAM CROCKER, and to Mr. W. S. BALLARD, U.S. Department of Agriculture, for valuable suggestions in regard to the apparatus for extracting the gas, and for many helpful suggestions during the progress of this work.

U.S. DEPARTMENT OF AGRICULTURE
WATSONVILLE, CAL.

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2. AUBERT, E., Nouvel appariel de MM. G. BONNIER et L. MANGIN pour l'analyse des gaz. Rev. Gen. Botanique **3**:97–104. 1894.
3. DENNY, F. E., Permeability of certain plant membranes to water. BOT. GAZ. **63**:373–397. 1917.
4. GRAFE, V., Ernahrungsphysiologisches Practicum höherer Pflanzen. p. 377. 1914.
5. GERBER, C., Recherches sur la maturation des fruits charnus. Ann. Sci. Bot. VIII. **4**: 1–280. 1896.
6. GORE, H. C., Studies on fruit respiration. U.S. Dept. Agric. Bur. Chem. Bull. no. 142. 1911.

BRIEFER ARTICLES

USE OF DILATOMETER IN STUDYING SOIL AND PLANT RELATIONSHIPS

The dilatometer was used successfully by BOUYOUCOS[1] in studying the forms of water in soils, and in studying the freezing point lowerings of soils and plants, MCCOOL and MILLAR[2] observed that the time of day when samples of plants were taken markedly influenced the freezing point lowerings of the leaves. The density of the sap was found to increase from morning until noon, and again decrease in the afternoon, reaching its lowest point at night. By means of the dilatometer the amount of water that froze readily was less at noon than in the morning, the freezing point lowerings apparently being governed somewhat by the form of water in the tissues.

We have repeated some of the experiments that were previously reported, and obtained additional information by means of the dilatometer. There are certain precautions that should be taken in making determinations of the amount of water that freezes in plant tissues by means of the dilatometer.

The sample is quickly weighed, inserted into the bowl of the dilatometer, and ligroin added. It is advisable to remove air bubbles either by shaking or by means of a suction pump. Where a bath of about $-1.5°$ C. is employed, 10 gm. of the tissue may be used; but with colder baths, such as -4 or $-6°$ C., it is imperative that much smaller quantities of tissue be added to the dilatometer, in order that supercooling may be brought about. With some plants 2 gm. are ample, while with others somewhat larger amounts may be employed to advantage. It is very difficult to accomplish supercooling if the freezing mixture or the dilatometer is agitated while the temperature of the material is being lowered. When equilibrium has been attained, solidification may be accomplished readily by agitating the dilatometer.

Several plants have been employed, namely, rye, wheat, corn, sweet clover, and red clover. They were grown on fertilized and untreated

[1] Tech. Bull. no. 36, Mich. Exper. Sta.

[2] The water content of the soil and the composition and concentration of the soil solution as indicated by the freezing point lowerings of the roots and tops of plants. Soil Sci. 3:113–138. 1917.

soils. The amounts of water that froze at different temperatures are reported in table I.

TABLE I

AMOUNT OF WATER FREEZING IN LEAVES OF PLANTS AT DIFFERENT TEMPERATURES

Crop	Date	Weight of material (gm.)	Freezing point lowering	Cc. water that froze		
				−1.5° C.	−4° C.	−6° C.
Rye.....	Nov. 24	5	0.928	0.90	2.50	.....
Rye.....	May 17	5	1.030	0.86	2.40	2.90
Wheat.....	Nov. 24	5	1.107	0.40	2.65	.....
Sweet clover.	Nov. 24	5	0.906	1.22	2.86	.....
Red clover...	May 15	5	0.780	1.70	2.70	2.90
Corn.....	June 10	5	0.578	2.10	2.40	.....

Marked variations in the amount of water that froze at −1.5° C. were observed, and while the amount increased with the lower temperatures, less differences were recorded.

The effect of the concentration of the soil solution and the water content of the soil were determined. The density of the solution in the soil was increased and so maintained by additions of the three salt solutions of SHIVE and the soil placed in 3 gallon jars and plants grown therein. The containers were placed in the open and exposed except when it rained.

Marked increase in the density of the soil solution resulted in little if any effect on the amount of water that froze at −2.5° and −4° C. respectively. The results are not given, inasmuch as MILLAR is to report them in another article.

The amount of water in the soil affected the quantity of water that froze in the plants grown therein. Corn was grown 60 days in sandy loam soil containing 9.5 and 15.6 per cent water respectively; 44.1 per cent of the loss in weight of the corn upon drying, froze at −2.5° C., and 63.9 per cent at −4° C. in the former and 51.8 and 84.8 per cent respectively in the latter. Similar results were obtained with barley.

In another series the moisture content of the soil was varied, but the concentration of the soil solution was kept about the same throughout the experiment by additions of the nutrient solution. In case of the higher temperature the results obtained were the reverse of those just given, or the amount of water that froze in the corn plant increased with a decrease in the water content of the soil when the nutrients were added. Slight differences were observed when the tissue was exposed to a temperature of −4.5° C.

This raises the question as to the effect of the composition of the soil solution upon the amount of easily freezable water in plant tissue. Possibly we shall be able to present results of experiments dealing with this question at a later date. It seems that the amount of water that readily freezes in the roots, stems, leaves, fruits, and seeds of plants and the factors that affect the freezing should be of general interest, at least to the physiologist, and it is probable that a knowledge of it would be valuable especially where the changes in the concentration of the cell contents of plants as well as winter injury are being investigated.

The difficulty encountered in causing tissues to solidify at the higher temperatures, especially when small amounts are used in the determinations, raises some important questions relative to winter injury of plants grown in different soils. It is possible and probable that some soils do not solidify, although the temperature may go appreciably below the freezing point. It is very easy to cause a sandy soil to solidify without much supercooling; with clay it is more difficult; while it is far more difficult in case of muck or peat. Instances have been observed where fruit trees growing in sandy soils have been severely injured by low temperature, while those growing in adjacent soils largely escaped. It is true, however, that sandy soils are more responsive to air changes than are the finer textured ones.—M. M. McCool and C. E. Millar, *Michigan Agricultural College, East Lansing, Mich.*

ISOLATING SINGLE SPORES

(WITH ONE FIGURE)

A new method of isolating single spores has been devised, which differs from other methods in common use in the substitution of a mechanical method of marking the location of the spores in the poured plates for the usual procedure of marking with ink-dots under the microscope. A cylinder of brass about the length of the ordinary 1.9 mm. objective is turned in the form shown in fig. 1, one end being provided with a thread like that of the objectives of the microscope to be used, and the other turned down and the end hollowed out so as to form a tube of the size desired.

This device is then screwed into the revolving nosepiece of the microscope in place of one of the objectives. The cover is now removed from the Petri dish containing the poured plates, and the spores are located under the microscope. When a spore is located with the objective, the tip of the marker is sterilized by flaming it with a gas burner or alcohol lamp, the nosepiece is rotated so as to bring the marker

over the spore in the place of the objective, and the marker is lowered so as to cut out a disk of agar inclosing the spore. The spore may then be examined again with the objective to see that it is really included in the agar disk, after which the disk is lifted from the Petri dish with a flattened platinum wire and placed in a culture tube. This method has the advantage of allowing a rapid and accurate location of spores and of guaranteeing that only a single spore is transferred. The disadvantages of exposing the culture to the air for rather long intervals is not a great one, because contaminations need not occur if proper precautions are taken. For such work the ringed tip of the marker may have a diameter of about 5 mm.

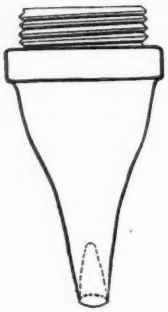

FIG. 1

A modification of this method has been found of great use in the selection of spores of *Pestalozzia*. In this case it was necessary, not only to locate single spores accurately, but also to measure the spores when located, so that the longest and the shortest might be taken. Finally it was found that this could be done by making dilution cultures, and then spreading the agar of the tube containing the desired dilution in a thin film on sterile glass slides. These slides could then be examined under the microscope, the spores measured, and those of the size desired cut out by the marking device, and finally the disks containing the spores could be transferred to culture tubes.

The agar for making the dilution cultures needs to be very carefully filtered so as to be as transparent as possible. For the work with *Pestalozzia* a 1 per cent solution of Liebig's beef extract with 3 per cent agar was used and found satisfactory. The nutrient solution, of course, must be of such a nature as to be suited to the fungus in question. The agar film on the slides is formed by pouring two or three drops of agar on the slide and then spreading them over the entire surface with a sterile needle. Too thin a film cannot be lifted from the slide; too thick a film will not allow the high power objective to be used, which is necessary with measurements of very small spores, so that some practice is needed to secure good results. I am indebted to Miss BACHMANN[3] for the idea of using agar films on slides, although films formed according to her method are too thin for this purpose. In this selection work a marker with a tip 1.5 mm. in diameter was found most useful.—CARL D. LARUE, *Kisaran, Asahan, Sumatra.*

[3] BACHMANN, FREDA M., Amer. Jour. Bot. **5**:32–35. 1918.

CURRENT LITERATURE

NOTES FOR STUDENTS

Taxonomic notes.—BECCARI[1] has monographed the palms of the Philippine Islands, recognizing 129 species in 22 genera, besides numerous varieties. Only 15 of these species have a wide distribution, and 5 are naturalized, leaving 109 endemic species. The new species are 10 in number, and *Adonidia* is established as a new genus. Much the largest genus is *Calamus*, with 36 species.

EVANS[2] has monographed the North American representatives of *Asterella*, "a difficult group of liverworts." A discussion of the morphological features of the genus is followed by a detailed taxonomic treatment of the 14 species, 3 of which are new.

PENNELL[3] has begun a series of papers dealing with the Scrophulariaceae of Wyoming, Colorado, and Utah, and of Idaho west to the 113th meridian, based upon extensive field work and also upon critical study of herbarium material. The purpose is to give for each species its taxonomic history, its flowering season, and its distribution. The first paper deals chiefly with *Pentstemon*, in which 88 species are recognized and arranged under 19 sections, 27 of the species being described as new.

BRAUSE,[4] in his study of Papuan Pteridophytes, describes 45 new species, chiefly in *Polypodium* (19), *Vittaria* (6), *Angiopteris* (5), and *Selaginella* (10). Of the 970 species of Pteridophytes listed, 597 are said to be endemic.

BROTHERUS,[5] in describing WEBERBAUER's collection of South American mosses, describes 29 new species.

PILGER,[6] in continuing his studies of South American grasses, describes 9 new species.

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[1] BECCARI, O., The palms of the Philippine Islands. Philippine Jour. Sci. **14**: 295–362. *pls. 3.* 1919.

[2] EVANS, A. W., The North American species of *Asterella*. Contrib. U.S. Nat. Herb. **20**:247–312. 1920.

[3] PENNELL, F. W., Scrophulariaceae of the Central Rocky Mountain States. Contrib. U.S. Nat. Herb. **20**:313–381. 1920.

[4] BRAUSE, G., Bearbeitung der von C. LEDERMANN von der Sepik-Expedition 1912 bis 1913 und von anderen Sammlern aus dem Papuagebiete früher mitgebrachten Pteridophyten, nebst Übersicht über alle bis jetzt aus dem Papuagebiet bekannt gewordenen Arten derselben. Bot. Jahrb. **56**:161–250. 1920.

[5] BROTHERUS, V. F., Musci Weberbaueriani. Beibl. Bot. Jahrb. **56**:1–22. 1920.

[6] PILGER, R., Gramineae austro-americanae imprimis Weberbauerianae. V. Beibl. Bot. Jahrb. **56**:23–30. 1920.

RIDLEY[7] has described two new genera of Malayan plants, namely *Peripetasma* (Menispermaceae) and *Scaphocalyx* (Flacourtiaceae).

MINOD[8] has made a very detailed study of the American species of *Stemodia* (Scrophulariaceae), recognizing 30 species, 5 of which are described as new. Thirteen species are eliminated from the genus, and in this connection the following new genera are established: *Chodaphyton*, *Lendneria*, *Verena*, and *Valeria*.

SMITH[9] has segregated a new genus (*Whytockia*) from *Stauranthera*, naming it for Mr. JAMES WHYTOCK, president of the Botanical Society of Edinburgh. It occurs in mountain forests of western China (Yunnan). The statement is made that recent collections show that southern and western China is rich in Gesneraceae.

Miss CURRIE[10] has published a critical study of Myxomycetes, chiefly from Ontario, representing 117 species and varieties, included in 29 genera. In several cases she has uncovered new facts as to morphology, physiology, and distribution. In the list of species and varieties are included 47 not previously recorded from Ontario, 36 new to Canada, and 5 new to North America. The plasmodia of two species were observed for the first time, a number are recorded as parasitizing fungi, and others as fruiting on leaves or stems of grasses or herbs to which they are injurious in some cases. The contribution represents a valuable addition to our knowledge of this interesting group.

HITCHCOCK and Mrs. CHASE,[11] in continuation of their studies of North American grasses, have published a revision of four genera of Paniceae, with full descriptions, details of distribution, and analytical keys. *Ichnanthus* is tropical American and includes 10 species, 5 of which have been published recently by the authors. *Lasiacis* is also tropical American, one species extending into Southern Florida. It was segregated from *Panicum* some years ago by HITCHCOCK, and is now recognized to include 15 species, one of which is described as new. *Brachiaria* extends into the southern United States and includes 5 species, one of which is new. *Cenchrus*, widely distributed, includes 13 species. The first two genera are revised by HITCHCOCK, and the last two by Mrs. CHASE.—J. M. C.

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[7] RIDLEY, H. N., New Malayan plants. Jour. Botany **58**:147–149. 1920.

[8] MINOD, MARCEL, Contribution a l'étude du genre *Stemodia* et du groupe des Stémodiées en Amérique. Thèse no. 606. Institut de Botanique, Univ. Genève. pp. 103. *figs. 41*. 1918.

[9] SMITH, W. W., *Whytockia*, a new genus of Gesneraceae. Trans. and Proc. Bot. Soc. Edinburgh **27**:338, 339. *pl. 7*. 1919.

[10] CURRIE, MARY E., A critical study of the slime-molds of Ontario. Trans. Roy. Can. Inst. **12**:247–308. *pls. 8–10*. 1919.

[11] HITCHCOCK, A. S., and CHASE, AGNES, Revisions of North American grasses. Contrib. U.S. Nat. Herb. **22**:1–71. *pls. 24. figs. 20*. 1920.

Chemical stimulation.—STEINBERG[12] has reported on further studies on the chemical stimulation of growth in *Aspergillus niger*, especially as brought about by Zn and Fe salts. Pfeffer's nutrient solution was used as the basis of all cultures. Commercial reagents, without further purification, were used in making solutions. It was found that 0.1 to 1.0 mg. Zn per liter was sufficient to furnish maximum stimulation, the growth thus stimulated averaging about four times that of the check. Successively lessening the amounts of each of the inorganic constituents of the solution resulted in all cases in decreased yield, showing none of them present in toxic or super-optimum concentration. Increasing the concentration of the various salts stimulated growth, although to a much less extent than that caused by Zn. Decreasing the acidity by adding alkalis decreased growth and gave strong spore formation. Increasing the H ion concentration by adding various acids stimulated vegetative growth and decreased spore formation. The type of growth resulting from increasing H ion concentration was very similar to that following Zn stimulation, but was always less in amount. STEINBERG believes the H ion concentration of Pfeffer's solution to be sub-optimum for *A. niger*, and suggests that the increased acidity of the solution with the addition of salts of the heavy metals with strong acids may be a very important factor in the stimulation to greater growth.

The Pfeffer solution was purified by autoclaving with $CaCO_3$ to precipitate Zn and other bivalent metals. Growth in such solutions was much less than in those not purified. Either Zn or Fe added alone stimulated growth somewhat, but not nearly so much as when both were added. Growth in the latter case was equal to that in any of the non-purified Zn stimulated cultures. STEINBERG believes previously secured stimulation from Zn alone to have been in the presence of Fe impurities.

No analyses were made to determine the chemical changes associated with the marked variation in growth rate and form brought about by the variation in the nutrient solution. Such data would be most interesting. It is unfortunate that reagents were not sufficiently repurified to eliminate entirely the possibility of Zn, Fe, Ni, and other such metals in the basic nutrient solution.—J. R. MAGNESS.

Mangroves.—Among the interesting features of a study of the red mangrove, *Rhizophora Mangle*, by BOWMAN,[13] is a synopsis of the historical development of our knowledge of the tree. The literature dates from 325 B.C. to the present, and includes references by THEOPHRASTUS, PLINY, PLUTARCH, BAUHIN, RAY, LINNAEUS, and LAMARCK, as well as more recent writers.

[12] STEINBERG, R. A., A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Amer. Jour. Bot. **6**:330–372. 1919.

[13] BOWMAN, H. H. M., Ecology and physiology of the red mangrove. Proc. Amer. Phil. Soc. **56**:589–672. *pls. 9*. 1917.

The investigations of BOWMAN were in the Dry Tortugas, and include many details regarding the morphology and structure of the various organs of the tree, including development and growth rate of the viviparous embryos. Measurements of the latter show a 4.7 cm. elongation of the emerging hypocotyls in 34 days. The results of transpiration studies show a lower rate of water loss with higher concentrations of sea water. The red mangrove is facultative in its growth in fresh and salt water, but requires the latter for optimum development. At least 2000 sq. miles of the tidal flats of the Philippine Islands are occupied by mangrove forests. The floristic, ecological, and economic characteristics of these forests of the sea have been described by BROWN and FISCHER.[14] Keys are provided for the recognition of the 30 principal species belonging to 16 different families. In addition to the well known aerial roots and viviparous habit of the mangroves, some of the notable features of these woodlands are the scanty undergrowth, the fairly numerous epiphytes, the myrmecophilous plants, and the frequent fringing of Nipa palms.

While the original stands of this forest contain trees of fair size yielding hard cabinet woods of excellent quality, the greater portion of the area is important only for the production of a good quality of firewood and for tan bark. The Nipa palm is important for alcohol production, and seems to present a possibility of utilization for sugar. Some cultivation of both the mangroves and the Nipa palms has proved successful; the former has also been used with good results in planting dykes and embankments to prevent the erosive action of the sea.—GEO. D. FULLER.

Age and area hypothesis.—The development of this hypothesis by WILLIS has been noted in this journal,[15] and now an analysis of the flora of New Zealand seems to strengthen his contentions.[16] The evidence in favor of the majority of endemics being of recent origin rather than relics is rather convincing.

Recently a floristic study of the plants of Stewart Island[17] yielded results supporting the hypothesis of the families and genera being represented in proportion to the number of genera and species respectively contained in them in New Zealand. The oldest forms are best represented in the flora, and the endemics are in the largest (in general, oldest) families and genera of New Zealand.

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[14] BROWN, WM. H., and FISCHER, A. F., Philippine mangrove swamps. P.I. Dept. Agric. and Nat. Res., Bur. For. Bull. **17**:132. *pls. 47*. 1918.

[15] BOT. GAZ. **61**:82. 1916; **62**:160. 1916; **63**:419. 1917; **64**:263. 1917; **65**: 116–117, 486. 1918.

[16] WILLIS, J. C., The sources and distribution of the New Zealand flora, with a reply to criticism. Ann. Botany **32**:339–367. 1918.

[17] ———, The flora of Stewart Island (New Zealand): a study in taxonomic distribution. Ann. Botany **33**:23–46. 1919.

The other islands about New Zealand also supply similar data.[18] In studying these floras WILLIS contends that through this hypothesis one is able to prophesy that the plants which reach outlying islands will be on the whole the oldest, and therefore the most widespread upon the mainland, and finds, on examining the facts, that the prophecy is completely fulfilled. The facts presented seem to support the contention and lead the author to restate the hypothesis thus: "The area occupied at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as the conditions remain reasonably constant, upon the age of the species of that group in that country, but may be enormously modified by the presence of barriers such as seas, rivers, mountains, change of climate from one region to the next or other ecological boundaries, and the like, also by the action of man, and by other causes. In other words, age and area is the chief positive, the action of barriers the chief negative, factor in plant distribution, while in recent times the action of man has become of greater importance than either."—GEO. D. FULLER.

Gases and germination.—KIDD[19] has studied the effect of various partial pressures of carbon dioxide and oxygen upon the sprouting of potatoes, and concludes that "(1) Oxygen is harmful to the potato tuber in concentration of about 5–10 per cent; oxygen 80 per cent kills in 4–5 weeks; oxygen 5–10 per cent is the optimal concentration for sprouting. (2) The harmful action of oxygen is increased in the presence of carbon dioxide. (3) Carbon dioxide inhibits sprouting in a concentration of 20 per cent. This concentration is at the same time to some extent harmful. (4) Higher concentrations of carbon dioxide cause marked injury and death." NOBOKIRCH has found that actively growing plant organs grow faster in oxygen pressures considerably below that of the normal atmosphere, but that such reduced pressures finally prove injurious, due to accumulation of metabolic products; while at normal oxygen pressures no such injury occurs. This may throw in question KIDD's interpretation that pressures above 10 per cent are injurious, especially for pressures up to the normal atmosphere. In general, due to their coats and other coverings, seeds are reduced in rate and percentage of germination by any reduction of oxygen pressure below the normal atmosphere, and often favored by greater oxygen pressures. Some of the work of APPLEMAN has indicated that oxygen supply is a limiting factor to germination of the potato, quite in contrast with KIDD's results. It is interesting that carbon dioxide showed no forcing action due to its anaesthetic properties. It is possible that it did cause increases in respiration, while not increasing or

[18] WILLIS, J. C., The floras of the outlying islands of New Zealand and their distribution. Ann. Botany **33**:267–293. 1919.

[19] KIDD, FRANKLIN, Laboratory experiments on the sprouting of potatoes in various gas mixtures. New Phytol. **18**:248–252. 1919.

even injuring growth as do some other anesthetics with the potato. In work of this kind one should be very sure that the gases used carry no other injurious gases.—WM. CROCKER.

Modification of unit characters.—An epoch in the perennial controversy between "mutationists" and "selectionists" is marked by CASTLE'S[20] shift from the latter to the former school. This investigator has previously held a leading place among "selectionists," with his modification by selection of the hooded character of rats. His change in point of view has been effected mainly by some of his own results. The cross between his plus race (+3.73) of hooded rats and a wild race brought a reduction in the grade of the hooded character as it appeared in the extracted hooded F_2 young. Repeated recrossing of these extracted individuals with the wild race finally resulted in extracted hooded rats of the grade +3.04. CASTLE concluded that the hooded character had been modified to this degree by its successive contacts with the germ plasm of the wild race. More recently he crossed his minus race (−2.63) with the same wild race. Repeated F_2 extractions showed successively the grades −0.38, +1.01, +2.55, and one family reached +3.05. These results indicate clearly that the hooded character in the plus and minus races are identical, only the multiple modifying factors differing. Repeated crosses with the wild race eventually produced hooded individuals whose quota of modifying factors approximated that of the wild race, evidently represented by a grade of +3.04 or +3.05. Consistent with the idea of a single unit for the hooded character and multiple modifying factors, the successive hooded populations that were extracted showed a decreasing degree of variability.—M. C. COULTER.

Rainfall efficiency.—The well known fact that plant foliage intercepts a considerable amount of the rainfall has been emphasized recently by MCLEAN[21] and others. A decidedly valuable contribution to the subject is represented by the extensive data of HORTON,[22] who has shown that the average observed interception during the summer of 1918 was 40 per cent of the precipitation. This loss ranges from 25 per cent for rains of long duration to 100 per cent for light showers, and seems to be nearly the same for most broad-leaved trees during the summer. These interception losses are greater for needle-leaved trees than for broad-leaved ones. Although the data are still insufficient to make an accurate comparison of the losses occurring at different seasons of the year, it is clear that since light showers are most frequent during the summer season the losses will be greatest during such a period, or in other

[20] CASTLE, W. E., Piebald rats and the theory of genes. Proc. Nat. Acad. Sci. 5:126–130. *fig. 1.* 1919.

[21] MCLEAN, R. C., Studies in the ecology of tropical rain-forest. Jour. Ecol. 7:121–172. 1919.

[22] HORTON, R. E., Rainfall interception. Mo. Weather Rev. 47:603–623. *figs. 17.* 1919

words, the efficiency of precipitation is least during the period of greatest need. The interception by full grown field crops is comparable in value with that from trees.

Selections from previously collected data and a short bibliography add their value to HORTON's report.—GEO. D. FULLER.

Hawaiian Lobelioideae.—ROCK[23] has published an elaborately printed and profusely illustrated monograph of the Hawaiian Lobelioideae. The very numerous photographic reproductions make the monograph almost equivalent to an herbarium set of the material. The tribe represents the family Lobeliaceae as ordinarily presented, and in the Hawaiian Islands includes 7 genera, 6 of which are endemic. The author has been studying this group for nearly 10 years, and has increased the 58 species of HILLEBRAND's *Flora* to 104, all peculiar to the Islands; and in his opinion many more species will be brought to light, especially in the genus *Cyanea*, which in the monograph includes 52 species. The only genus of world-wide distribution is *Lobelia*, which is credited with 11 species in the Islands, 4 of which are new. The first part of the monograph contains a general discussion of structure, habit, and distribution. —J. M. C.

Evolution of cotyledony.—BUCHHOLZ[24] has investigated the ontogeny of the cotyledons in a number of living conifers, and has reached some important conclusions. He finds in certain conifers a considerable number of primordia, which may develop a corresponding number of cotyledons, or fusions may occur, thus reducing the number. In no case was there any evidence of increasing the number of cotyledons by splitting. The fusions resulting in a reduced number of cotyledons in some cases resulted also in cotyledonary tubes. The inference is that polycotyledony is primitive; that dicotyledony was attained by a general fusion of many cotyledons into two groups or by a bilabiate development of the cotyledonary tube; and that monocotyledony is the result of a cotyledonary tube becoming unilabiate. This evolutionary sequence seems to be borne out by all the facts at hand, and relates the different forms of cotyledony in a natural way.—J.M.C.

Proceedings of the Indiana Academy.—The volume of Proceedings of the Indiana Academy of Science for 1918 has just been distributed, including 327 pages and numerous illustrations. Among the botanical contributions published are the following: The barberry and its relation to the stem rust of wheat in Indiana, F. J. PIPAL; A method of teaching diffusion and osmosis

[23] ROCK, JOSEPH F., A monographic study of the Hawaiian species of the tribe Lobelioideae, family Campanulaceae. Publ. Bernice Pauahi Bishop Museum. 4to pp. xvi+395. *pls. 217*. 1919.

[24] BUCHHOLZ, JOHN T., Studies concerning the evolutionary status of polycotyledony. Amer. Jour. Bot. **6**:106–119. *figs. 25*. 1919.

in connection with biological work, PAUL WEATHERWAX; Bacteria in frozen soil, H. A. NOYES; Some abnormalities in plant structure, M. S. MARKLE; Plants of Boone County, Kentucky, JAMES C. NELSON; Plants new to Indiana. VIII, CHARLES C. DEAM; Analyses of 100 soils in Allen County, Indiana, R. H. CARR and V. R. PHARES; The relation of nitrogen, phosphorus, and organic matter to corn yield in Elkhart County, Indiana, R. H. CARR and LEROY HOFFMAN; Soil survey of Cass County, Indiana, COLONZO C. BEALS; Ascomycetes new to the flora of Indiana, BRUCE FINK and SYLVIA C. FUSON; The dormant period of timothy seed after harvesting, M. L. FISHER.—J. M. C.

Douglas firs.—HENRY[25] and FLOOD have described three American and four Asiatic species of *Pseudotsuga*, separating the Pacific Coast trees from those found in the Rocky Mountains. Aside from some minor differences in leaf and cone structure, the authors believe that the Rocky Mountain form, *P. glauca*, shows more xerophytic structures and is much more resistant to injury by frost and drought. The differences in the behavior of the two forms under silvicultural conditions in Great Britain seems to afford a much better basis for considering the eastern form a separate species. Of the Asiatic species, one is native to Japan, one to Formosa, and two are native to Yunnan, China. All are found in restricted areas and are to be regarded as so rare as to be of little economic importance.—GEO. D. FULLER.

Evaporation and vapor pressure deficit.—It has been shown by JOHNSTON[26] that it is possible, by using vapor pressure deficit and wind velocity data, the former being derived from hygrometer and thermometer readings, to calculate the "potential evaporation" or evaporating power of the air in a manner that will show a very close agreement with the records from the porous cup atmometer. In this way considerable data collected by the Weather Bureau may be translated into terms that are significant and valuable for the ecologist.—GEO. D. FULLER.

Plantago in Hawaii.—ROCK[27] has monographed the two endemic species of *Plantago* occurring in the Hawaiian Islands. One of them, *P. princeps*, is a branching shrub, and its variability is indicated by the fact that 8 varieties are recognized. The other species, *P. pachyphylla*, includes 7 varieties, among which there is a new one (var. *anomala*) which combines the characteristic capsule and venation of *P. pachyphylla* with the seeds and arborescent branching habit of *P. princeps*.—J. M. C.

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[25] HENRY, A., and FLOOD, MARGARET G., The Douglas firs: a botanical and silvicultural description of the various species of *Pseudotsuga*. Proc. Roy. Irish Acad. **35**: sect. B. 67–92. *pls. 12–14.* 1920.

[26] JOHNSTON, E. S., Evaporation compared with vapor pressure deficit and wind velocity. Mo. Weather Rev. **47**:30–33. *figs. 2.* 1919.

[27] ROCK, J.F., The genus *Plantago* in Hawaii. Amer. Jour. Bot. **7**:195–210. *pl. 13.* 1920.